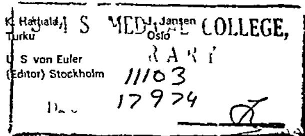
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Effects of Acetylcholine and Some Other Smooth Muscle Stimulants on the Electrical and Mechanical Responses of the Guinea-Pig Vas Deferens to Nerve Stimulation

By

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Abstract

SJÖSTRAND N O *Effects of acetylcholine and some other smooth muscle stimulants on the electrical and mechanical responses of the guinea pig vas deferens to nerve stimulation* Acta physiol scand 1973 *89* 1—9

The effect of acetylcholine (Ach) histamine, 5-hydroxytryptamine Ba^{2} and bradykinin on the electrical and mechanical responses of the guinea pig vas deferens to nerve stimulation was investigated with the sucrose gap technique The agonists produced a depolarization of the smooth muscle membrane Because of this the membrane came closer to the threshold for firing of action potentials (AP s) The AP s also increased in size in the presence of the agonists and the contractions became larger Atropine abolished the effect of Ach The results indicate that the agonists enhance the motor response of the vas deferens by a direct action on the smooth muscle cell membrane

Low concentrations of acetylcholine (Ach) which by themselves do not contract the guinea pig vas deferens cause a conspicuous enhancement of the motor response of the organ to sympathetic nerve stimulation (Sjöstrand 1961 Yamamoto and Nakanishi 1964 Sjostrand and Swedin 1968 Euler 1970 Saxena 1970) This effect is blocked by atropine and is not of a preganglionic origin Since a number of other smooth muscle stimulants *e g* histamine serotonin (5 HT) Ba and bradykinin exert a similar action (Sjostrand 1961 Sjostrand and Swedin 1968 Saxena 1970) it has been suggested (Sjostrand and Swedin 1968) that Ach and the other agonists mainly act on the smooth muscle cells On the other hand it has also been suggested that Ach could act on the nerve terminals and increase the amount of released transmitter on nerve stimulation (Euler 1970)

The present investigation was performed in order to obtain information about the electrical as well as the mechanical effects of the smooth muscle stimulants on the vas deferens It was hoped that an electrophysiological analysis might elucidate the mechanisms by which low concentrations of Ach and other agonists augment the

motor responses of the organ to nerve stimulation A preliminary account of some of the results has already been presented (Sjostrand 1971)

Methods

The sucrose gap technique (Burnstock and Straub 1958 Bulbring and Burnstock 1960) as modified by Burnstock Holman and Kuriyama (1964) was used

The guinea pigs were stunned and bled The vas deferens was dissected free from loose connective tissue but precaution was taken not to destroy the nerves running outside the organ This because extensive cleaning and stripping of the vas deferens was found to diminish the size of the excitatory junction potentials and to induce instability of the resting potential and spontaneous activity of the organ A thread was tied around the vas deferens and the supplying nerves just below the peripheral ganglia (*cf* Sjöstrand 1965 and Ferry 1967) The part of the organ located above the knot as well as the peripheral ganglia were cut away The organ was placed in a sucrose gap apparatus having 2 stimulating electrodes 2 mm apart The Ag electrodes were located about 3 mm from the knot and the distance from the electrodes to the junction between the sucrose and the warmed Krebs solution was 8 mm The epidydimal end of the vas deferens was superfused with 1 19 % K_2SO_4 solution Mechanical recording was obtained from the prostatic end which was superfused with Krebs solution at 34—36 C and bubbled with 5 % CO_2 in 95 % O_2 The tissue was stretched lightly (load 0 2—0 5 g) Nerve stimulation was applied by square wave pulses of 0 1—2 ms duration In most experiments *the duration was 0 5 ms The voltage was in the range 10—50 V When repetitive stimuli* were given the frequency was always kept within the physiological range *i e* not exceeding 15 Hz (Folkow 1952) Ag AgCl electrodes were used for the electrical recording and a battery driven isometric semiconductor strain gauge tensiometer for the mechanical recording

The following electrical apparatus were used a Nikon Kohden MZ-4 amplifier with corresponding cathode follower or an amplifier made in the Oxford laboratory a Hellige Helcoscripter He 86 with corresponding preamplifiers or a Grass Polygraph and a Grass S-4 stimulator with a stimulus isolation unit (SIU 5) The electrical and mechanical activity was also followed on an Oscilloscope (Tektronix 502 A) The presented figures are drawings from Hellige records

The Krebs solution had the following composition (mM) Na 137 K 5 9 Mg^{2} 1 2 Ca^{2} 2 5 Cl 134 H_2PO_4 1 2 HCO_3 15 5 glucose 11 5 The following drugs were used Acetylcholine chloride atropine sulphate hexamethonium bromide histamine dihydrochloride serotonin creatinine sulphate barium chlorid and bradykinin (BRS 640 Sandoz) in commercial solvent The concentrations of the drugs refer to the salts The present report is based on observations on 75 vasa deferentia from 59 albino guinea pigs weighing 400—700 g

Results

Low frequency (0 1—1 Hz) nerve stimulation evoked individual excitatory junction potentials (EJP) They showed facilitation and the maximal amplitude was generally reached within the fifth to tenth EJP Increase in stimulation frequency voltage or duration of the stimuli increased the size of the EJP s Summation of the EJP s was generally seen with frequencies exceeding 1 Hz and sometimes also with lower frequencies With frequencies exceeding 5 Hz it was difficult to distinguish the individual EJP s A compound action potential (AP) and contraction could be elicited by a single EJP in some preparations but in others summating EJP s were needed to reach the firing threshold These findings agree well with those of earlier investigators using intracellular microelectrodes or the sucrose gap technique (Burnstock and Holman 1961 Kuriyama 1963 Burnstock Holman and Kuriyama 1964 Furness and Burnstock 1969) In most preparations AP s obtained by a single EJP were small The contractions following these AP s were also generally small AP s obtained with frequencies of nerve stimulation causing summation of EJP s were

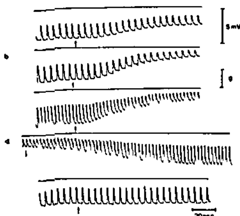


Fig 1 Sucrose gap recordings of subthreshold EJP s from a guinea pig vas deferens showing the effect of Ach Upper tracing mechanical recordings lower tracing electrical recordings Nerve stimulation 18 V 0 5 ms duration Frequency in *a* *b* and *e* 0 2 Hz in *c* and *d* 0 4 Hz *a* at arrow Ach 2×10⁻⁸ g/ml *b* at arrow Ach 5×10⁻⁸ g/ml *c* at arrow Ach 5×10⁻⁸ g/ml *d* continuous recording from *c* showing washing out of Ach *e* recording in the presence of atropine 1×10⁻⁶ g/ml At arrow Ach 5×10⁻⁵ g/ml

generally larger and the contractions were larger too With these frequencies the AP s usually increased in size with increasing frequency and the contractions also increased in magnitude To prolonged (5—15 s) stimulation at high frequency (3—15 Hz) the organ responded in general with a large initial AP followed by smaller AP s Maximal tension developed as a rule after the first large AP and thereafter the tension declined However in most experiments the electrical recordings were complicated by rather large contraction artefacts following the first spike Therefore most observations of responses to this type of stimulation were obtained in rather weak preparations using low voltage (10—20 V) for stimulation With larger voltages (30—50 V) and frequencies of 10—15 Hz the observation of Furness and Burnstock (1969) was confirmed that the maximal frequency of repetitive spikes in the guinea pig vas deferens is 1—2 per second

When the effects of drugs were investigated the following patterns of stimulation were applied 1 Single stimuli of varying voltage 2 Repetitive stimuli with a low frequency (0 1—1 Hz) giving non summating EJP s below or close to the threshold for firing of AP s 3 Repetitive stimuli of frequencies (1—15 Hz) inducing summating EJP s and an AP where the stimulation was interrupted as soon as the AP had appeared and 4 High frequency stimulation (3—15 Hz) continued for 5—15 s

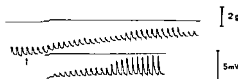


Fig 2 Sucrose gap recordings of subthreshold EJP s from a guineapig vas deferens Upper tracings mechanical activity lower tracings electrical activity Nerve stimulation duration 0 5 ms frequency 0.3 Hz 15 V and at dots 30 V At arrow in upper recordings Ach 1×10⁻⁸ g/ml Lower recordings control Note that the 30 V EJP s are decreased to a greater extent than the 15 V EJP s in the presence of Ach.

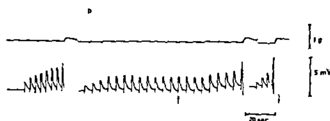


Fig 3 Sucrose gap recordings of EJP s and contractions from a guinea pig vas deferens Nerve stimulation 20 V 0 5 ms duration *a* at a frequency of 0 3 Hz a spike and contraction appears at the 8th EJP *b* at 0 2 Hz the threshold is not reached but with Ach 5×10^{-9} g/ml at arrow the preparation is depolarized and the EJP s induce a spike and contraction *c* when the preparation is stimulated at 0 3 Hz in the presence of Ach 5×10^{-9} g/ml threshold is reached at the 4th EJP

Effects of Acetylcholine

Ach in concentrations of 1×10^{-9}—1×10^{-6} g/ml produced a depolarization of the smooth muscle The depolarization was never large enough to reach the level for firing of AP s In order to obtain AP s and contractions concentrations exceeding 5×10^{-6} g/ml had to be used When Ach had produced a depolarization of the smooth muscle cells the size of evoked subthreshold EJP s diminished (Fig 1 and 2) A larger depolarization caused by a higher concentration of Ach diminished the EJP s slightly more than a smaller depolarization caused by a smaller concentration (Fig 1 a and b) Larger EJP s due to increased stimulation frequency or increase in voltage or duration of the stimuli were also relatively more reduced than smaller fully facilitated EJP s (Fig 1 b, c and Fig 2) The net sum of depolarization caused by Ach and the EJP s was however, always larger than that caused by the EJP s alone (Fig 1 and 2) Because of this EJP s which by themselves were too small to produce AP s could induce spikes and contractions in the presence of Ach This was especially easy to demonstrate in preparations where the EJP s were close to the firing level of spikes (Fig 3) When single stimuli were applied a spike and ontraction were obtained with a lower voltage in the presence of Ach than in its sence (*cf* Fig 7) It was however noted that prolonged exposure to Ach (10—15 min) gradually increased the threshold of some preparations and a greater voltage was now required in order to elicit a spike

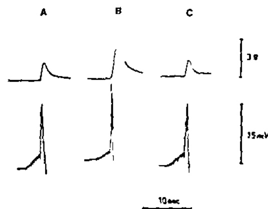


Fig 4 Sucrose gap recordings of summating EJP s AP s and contractions from a guinea pig vas deferens Nerve stimulation 25 V 0 5 ms duration ? Hz *a* control period before Ach *b* Ach 5×10^{-8} g/ml present (for 2 min) *c* control period 10 min after washing out Ach Note the smaller and split AP s in the control periods and the larger unsplit AP preceding the large contraction when Ach is present

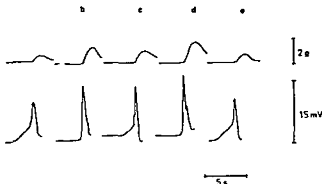


Fig 5 Sucrose gap recordings from a guinea pig vas deferens showing AP s and contractions Nerve stimulation 18 V 0 5 ms duration *a* response to 5 Hz *b* response to 15 Hz *c* 5 Hz in the presence of Ach 5×10^{-8} g/ml (for 2 min) *d* 15 Hz in the presence of Ach 5×10^{-8} g/ml (for 3 min) *e* 5 Hz 10 min after Ach The individual EJP s are not seen with these frequencies Note that the spikes elicited are larger and the contractions increase upon increasing frequency and in the presence of Ach

When the preparations were stimulated with frequencies inducing summation of the EJP s and eventually a spike and contraction less EJP s were generally required to initiate a spike (Fig 4) This was clear when the EJP s were large and the Ach induced depolarization was moderate but not so prominent when the EJP s were small and a rather high concentration of Ach was used

There was a change in configuration of the AP in the presence of Ach It became larger and in preparations where the AP was split it became less split (Fig 4) Simultaneously the contractions became larger The effect of Ach on the AP s emulated that of a stimulation with higher frequency (Fig 5) In general larger concentrations of Ach caused a larger AP and a larger contraction than smaller concentrations In many preparations there was also a shortening of the duration of the AP Due to the contraction artefacts it was however often difficult to evaluate this effect The above mentioned increase in threshold after prolonged exposure to Ach een in some preparations was further illustrated in experiments with summating EJP s since gradually more EJP s were needed to reach the threshold The effect of Ach on the AP s and the contractions persisted however after prolonged exposure to the drug

Upon prolonged repetitive stimulation inducing several AP s exposure to Ach led to an increased firing of spikes (Fig 6) and a greater tendency towards the pattern of repetitive spike firing described by Furness and Burnstock (1969)

Atropine (1×10^{-7}—1×10^{-6} g/ml) had no overt effect by itself on the electrical or mechanical response of the vas deferens to nerve stimulation Atropine abolished all effects of Ach on the preparations After atropinization (1×10^{-6} g/ml) not even concentrations of 5×10^{-5} g/ml of Ach had any effect on the preparations (Fig 1 e) *Hexamethonium* (1×10^{-5}—1×10^{-4} g/ml) had no effect on the EJP s and left the effects of Ach unimpaired

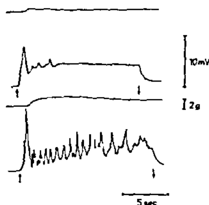


Fig 6 Sucrose gap recordings from a guinea pig vas deferens showing the response to continuous repetitive nerve stimulation (between arrows) 12 V 0 5 ms duration and 7 Hz. *a* Control The stimulation induces a large spike followed by 3 smaller spikes the tissue is still depolarized but no spike is elicited and contraction declines *b* In the presence of Ach 1×10^{7} g/ml (for 3 min) the first spike is larger and followed by several spikes and the contraction is larger

Effects of other smooth muscle stimulants

Histamine (1×10 —1×10 g/ml) also produced a depolarization of the vas deferens The effect of these concentrations of histamine on the response of the organ to nerve stimulation was very similar to that of Ach in all types of experiments In Fig 7 recordings are presented showing the effect of histamine on a preparation where it was possblie to induce spikes and contractions with single stimuli

Serotonin Similar results as with Ach were also obtained with 5 HT (1×10^{6}—2×10^{5} g/ml) The preparations were rather in ensitive to the drug and moreover there was a considerable variation in sensitivity among the preparations Some preparations also developed tachyphylaxis to 5 HT In one respect 5 HT differed slightly from Ach and histamine It seemed to raise the threshold for initiating APs more rapidly upon prolonged drug exposure

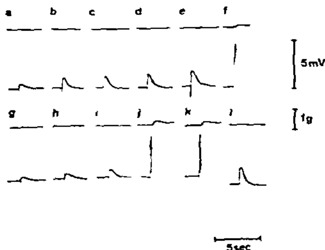


Fig 7 Sucrose gap recordings of EJP s spikes and contractions in a guinea pig vas deferens preparation where a single stimulus could induce AP s Recordings *a* to *f* control *g* to *k* in the presence of histamine 1×10^{6} g/ml (for 2 min) Nerve stimulation with pulses of 0 5 ms duration in *a* 10 V in *b* 15 V in *c* 20 V in *d* 25 V in *e* 30 V in *f* 35 V in *g* 10 V in *h* 15 V in *i* 20 V in *j* 25 V in *k* 30 V *l* is a control with 30 V recorded 15 min after removing histamine Stimuli applied every minute When the tissue is depolarized by histamine the EJP s are smaller but a lower voltage is needed for eliciting AP s from the EJP s

Barium chloride (5×10^{-5}—2×10^{-4} g/ml) also produced a depolarization of the vas deferens preparation The depolarization was often long lasting and persisted 15 min or more after returning to normal Krebs solution During the Ba^{2+} depolarization the effects on the response to nerve stimulation were similar to those of Ach

Bradykinin Some experiments were also performed with bradykinin (5×10^{-7}—2×10^{-6} g/ml) It produced similar effects as Ach In some preparations a reduction in size of the EJP s was seen after washing out the bradykinin This was probably due to the solvent of the bradykinin preparation which contains chlorbutol since the solvent alone was found to reduce EJP s

Discussion

The general effect of Ach and the other plain muscle stimulants on the guinea pig vas deferens is a depolarization of the smooth muscle cells of the organ The decrease in size of the EJP s seen during this depolarization is in accordance with findings from other systems of junctional transmission (*cf* Ginsborg 1967) and also with recent findings on the mouse vas deferens (Furness 1970) Although the EJP s are reduced during the depolarization the net sum of the depolarization caused by the EJP s and that caused by the agonist is larger than the depolarization caused by either stimulus alone This appearantly brings the smooth muscle cells of the tissue closer to the threshold for firing of AP s Hence subthreshold EJP s can elicit AP s and contractions if the tissue is simultaneously exposed to a depolarizing drug Therefore on nerve stimulation activating only a part of the muscle cells (or rather groups of muscle cells) more cells would be engaged in the contraction if an agonist is present Such an action of Ach and the other agonists is in accordance with the finding of Bell (1967) that high concentrations of choline which depolarize the guinea pig vas deferens decreased the stimulation voltage necessary to induce contractions of the organ

The increase in size of the AP s and the simultaneous increase in contraction tension seen in the presence of the agonists indicate that a larger area of smooth muscle cell is activated by nerve stimulation Such an interpretation is favoured by the increase in size of the compound AP s also seen on increasing the stimulation frequency This effect of the agonists on the AP s may be explained as a result of an increased engagement of smooth muscle cells brought closer to the threshold by the depolarizing agents However an additional event is probably a facilitated propagation of the AP s in the tissue AP s are propagated in the guinea pig vas deferens over a rather long distance (Burnstock Holman and Kuriyama 1964 Furness and Burnstock 1969) If the tissue is moderately depolarized less current would be needed in order to discharge the capacitance of the cell membranes This would favour the spread of excitation through the tissue Hence the excitation wave would reach more cells In this respect the finding that the increase in size of the AP and the contractions persisted also in preparations showing an increase in threshold after prolonged exposure to the agonists may be indicative This increase in threshold

seen in many preparations after prolonged exposure to Ach and the other smooth muscle stimulants probably reflects an accommodation of the smooth muscle to the long lasting depolarization

The shortening of the duration of the compound AP seen in some preparations in the presence of the agonists may indicate an increased conduction velocity and a greater synchronization of the activity in the preparation Such an effect of Ach has been described for the guinea pig taenia coli (Burnstock 1958) The change in spike configuration from split to unsplit spikes seen in some preparations when agonists were present also indicates a greater synchronization of the electrical activity in the organ

The present findings do not indicate that the Ach potentiation of the motor response of the vas deferens is due to an effect on the transmitter release in the organ An effect of Ach on adrenergic neurotransmission has been repeatedly suggested in the literature (*cf* Burn 1967, Muscholl 1970) This possibility has been investigated by quantitative determinations of the noradrenaline release from the rabbit heart in a number of studies by Lindmar Loffelholz and Muscholl (see review and summary by Muscholl 1970) According to these studies noradrenaline release is facilitated by a nicotinic action of Ach operating at high concentrations of the drug This effect has a fairly short duration On the other hand low concentrations of Ach inhibits the noradrenaline release through a muscarinic action In the present study even very large concentrations of Ach in atropinized preparations had *no effect* and in non atropinized preparations low concentrations of Ach had similar effects on the EJP s as the other agonists Further Ach enhances the motor response of electrically stimulated denervated vasa deferentia (Sjostrand and Swedin 1973) Thus the plausible explanation of the enhancement caused by Ach and the other agonists of the motor response of the vas deferens to nerve stimulation is an increased recruitment of smooth muscle cells in the contraction due to a partial depolarization of cells An additional effect on the contractile processes effectuated by *e g* Ca influx to the cells can of course not be excluded

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References

BELL C Effects of exogenous choline on adrenergic responses of the guinea pig vas deferens *Brit J Pharmacol* 1967 *30* 436—444

BULBRING E and G BURNSTOCK Membrane potential changes associated with tachyphylaxis and potentiation of the response to stimulating drugs in smooth muscle *Brit J Pharmacol* 1960 *15* 611—624

BURN J H Release of noradrenaline from the sympathetic postganglionic fibre *Brit med J* 1967 *2* 197—201

BURNSTOCK G The effects of acetylcholine on membrane potential spike frequency conduction velocity and excitability in the taenia coli of the guinea pig *J Physiol* (Lond) 1958 *143* 165—182

BURNSTOCK G and M F HOLMAN The transmission of excitation from autonomic nerve to smooth muscle *J Physiol* (Lond) 1961 *155* 115—133

BURNSTOCK G M E HOLMAN and H KURIYAMA, Facilitation of transmission from autonomic nerve to smooth muscle of guinea pig vas deferens *J Physiol* (Lond) 1964 *172* 31—49

BURNSTOCK G and R. W STRAUB A method for studying the effects of ions and drugs on the resting and action potentials in smooth muscle with external electrodes *J Physiol* (Lond) 1958 *140* 156—167

EULER, U S v Effect of some metabolic factors and drugs on uptake and release of catecholamines in vitro and in vivo In *Bayer-Symposium II* eds H J Schumann and G Kroneberg Berlin Springer 1970 144—158

FERRY C B The innervation of th vas deferens of the guinea pig *J Physiol* (Lond) 1967 *192* 463—478

FOLKOW B Impulse frequency in sympathetic vasomotor fibres correlated to the release and elimination of the transmitter *Acta physiol scand* 1952 *25* 49—76

FURNESS J B The excitatory input to a single smooth muscle cell. *Pflugers Arch ges Physiol* 1970 *314* 1—13

FURNESS J B and G BURNSTOCK A comparative study of spike potentials in response to nerve stimulation of the vas deferens of the mouse rat and guinea-pig *Comp Biochem Physiol* 1969 *31* 337—345

GINSBORG B L Ion movements in junctional transmission *Pharmacol Rev* 1967 *19* 289—316

KURIYAMA H Electrophysiological observations on the motor innervation of the smooth muscle cells in the guinea pig vas deferens *J Physiol* (Lond) 1963 *169* 213—228

MUSCHOLL, E Cholinomimetic drugs and release of the adrenergic transmitter In *Bayer Symposium II* eds H J Schumann and G Kroneberg Berlin Springer 1970 168—186

SAXENA P R. Effect of some drugs on the responses of the vas deferens and seminal vesicle to hypogastric nerve stimulation in guinea pig in vivo *Pharmacology* 1970 *3* 220—228

SJOSTRAND N O Effect of some smooth muscle stimulants on the motor response of the isolated guinea pig vas deferens to hypogastric nerve stimulation *Nature* (Lond) 1961 *192* 1190—1191

SJOSTRAND N O The adrenergic innervation of the vas deferens and the accessory male genital glands *Acta physiol scand* 1965 *65* Suppl 257

SJOSTRAND N O Effect of acetylcholine on the electrical and mechanical responses of the guinea pig vas deferens to nerve stimulation *Acta physiol scand* 1971 *82* 36A—37A

SJOSTRAND N O and G SWEDIN Potentiation by smooth muscle stimulants of the hypogastric nerve vas deferens preparation from normal and castrated guinea pig *Acta physiol scand* 1968 *74* 472—479

SJOSTRAND N O and G SWEDIN On the mechanism of the enhancement by smooth muscle stimulants of the motor responses of the guinea pig vas deferens to nerve stimulation *Acta physiol scand* To be published

YAMAMOTO H and H NAKANISHI Effects of ganglionic blocking agents on responses of ductus deferens preparation of guinea pig to hypogastric nerve stimulation *Jap J Pharmacol* 1964 *14* 256—267

Acta physiol scand 1973 89 10—18

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Effects of Adrenaline, Noradrenaline and Isoprenaline on the Electrical and Mechanical Responses of the Guinea-Pig Vas Deferens to Nerve Stimulation

By

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Abstract

SJÖSTRAND N O *Effects of adrenaline noradrenaline and isoprenaline on the electrical and mechanical responses of the guinea pig vas deferens to nerve stimulation* Acta physiol scand 1973 89 10—18

The effect of noradrenaline adrenaline and isoprenaline on the electrical and mechanical responses of the guinea pig vas deferens to nerve stimulation was studied with the sucrose gap technique A stimulatory α adrenergic effect was found This was due to a depolarization of the smooth muscle cell membrane An inhibitory β adrenergic effect was observed in many preparations It consisted of a hyperpolarization of the membrane potential and a decrease in size of the excitatory junction potentials Thirdly in a few preparations an inhibition of the excitatory junction potentials was seen which was not a β effect but could partly be antagonized by phentolamine It is suggested that this effect is due to an α sympathicomimetic inhibition of neurotransmission in the vas deferens

Different results have been obtained concerning the acute effects of exogenous adrenaline (A) and noradrenaline (NA) on the motor response of the isolated guinea pig vas deferens to nerve stimulation An augmentation of the contractile response has been reported by several authors (Sjöstrand 1961, Holman and Jowett 1964 Yamamoto and Nakanishi 1964 Large 1965 Sjöstrand and Swedin 1968, Saxena 1970) This effect is blocked by α blockers On the other hand an inhibitory action of the catecholamines has also been described (Holman and Jowett 1964 Large 1965 Hotta 196[illegible] Ambache and Zar 1971) The inhibitory action has been found to be blocked by β adrenergic blockers and mimicked by isoprenaline by some investigators (Holman and Jowett 1964 Large 1965) and therefore ascribed to an activation of β adrenergic receptors However this explanation has been denied by other investigators and the effect attributed to either a desensitization of junctional α receptors (Hotta 196[illegible]) or to an inhibition of the neurotransmission in the vas deferens (Ambache and Zar 1971) The latter investigators

have even questioned the role of noradrenaline as the main motor neurotransmitter in the vas deferens

The present investigation was performed in order to obtain electrophysiological information about 1) the stimulant action of the catecholamines on the response to nerve stimulation 2) the possible presence of inhibitory β receptors and 3) the possible existence of an inhibitory effect of the catecholamines on the response to nerve stimulation not mediated by β receptors

Methods

The sucrose gap technique (Burnstock and Straub 1958 Bulbring and Burnstock 1960) was used The details of the procedure have been described in a preceding report (Sjostrand 1973) a)

The following drugs were used l Noradrenaline bitartrate l adrenaline bitartrate l isoprenaline sulphate phentolamine mesylate and propranolol hydrochloride The concentrations of the drugs are expressed as salts

The present report is based on 59 vasa deferentia from 45 albino guinea pigs weighing 400—700 g

Results

The response of the smooth muscle in the vas deferens to field stimulation using different stimulus parameters to stimulate the nerves in the tissue are well known (Burnstock and Holman 1961 Kuriyama 1963 Burnstock Holman and Kuriyama 1964 Furness and Burnstock 1969) The particular stimulation patterns chosen for the investigation of the influence of excitatory agonists and the general responses observed are described in the preceding paper (Sjöstrand 1973)

Effects of noradrenaline and adrenaline

The two catecholamines produced a depolarization of the vas deferens This was seen with concentrations between 1×10^{8} to 5×10^{6} g/ml With lower concentrations no effect could be detected Concentrations exceeding 1×10^{5} g/ml usually produced spikes and contractions of the organ There was no consistent difference in sensitivity to the two catecholamines

During the depolarization by the catecholamines the individual excitatory junction potentials (EJP s) elicited by stimulation of 0 1—0 5 Hz diminished but in most of the preparations (48 out of 59) the net sum of the depolarization caused by the EJP s and the depolarization caused by the catecholamines was larger than that caused by either stimulus alone as seen in Fig 1 a In these preparations phentolamine 1×10^{6} g/ml abolished the depolarization caused by the catecholamines in concentrations up to 1×10^{6} g/ml (Fig 1 b) but when the catecholamine concentration was increased to 5×10 g/ml or more the phentolamine block was overcome and a depolarization could be recorded again (Fig 1 c) Propranolol 1—5×10^{6} g/ml enhanced the depolarization produced by the catecholamines clearly in about 1/3rd of the preparations (*cf* Fig 4 b) but not in the others In most preparations

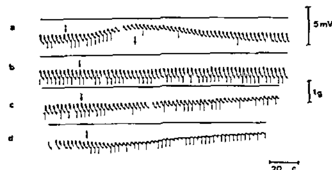


Fig 1 Sucrose gap recordings of EJP s from a guinea pig vas deferens Stimulation 25 V 0 5 ms duration 0 4 Hz *a* at first arrow noradrenaline 5×10^{7} g/ml washed out at second arrow *b* in the presence of phentolamine 1×10^{6} g/ml (for 5 min) at arrow noradrenaline 5×10^{7} g/ml *c* still in the presence of phentolamine 1×10^{6} g/ml at arrow noradrenaline 1×10^{5} g/ml *d* at arrow phentolamine 5×10^{5} g/ml

as shown in Fig 2 b less EJP s were also needed to obtain a spike unless the catecholamine concentration was increased so much (Fig 2 c) as to cause depolarization close to the threshold for firing of action potentials, (AP s) Moreover no suppression of the EJP s was seen when a high concentration of noradrenaline was present during phentolamine exposure (Fig 2 d—f)

When the preparations were stimulated with a high frequency (3—15 Hz) for a longer period (5—15 s) the catecholamines increased the firing of spikes and promoted the pattern of repetitive spike firing (Fig 3)

In 11 preparations it was noted that the catecholamines reduced the EJP s to a greater extent than that which could be expected from the depolarization so that the net sum of depolarization caused by the catecholamines and the EJP s was less than that caused by the EJP s alone (Fig 4 a) This effect could not be abolished by propranolol 1—5×10^{6} g/ml (Fig 4 b) Moreover when the depolarizing effect of the catecholamines had been abolished by phentolamine 1×10^{6} g/ml the size of the

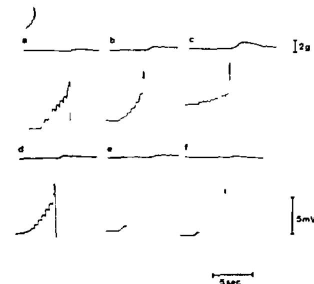


Fig 2 Sucrose gap recordings showing summating EJI s AP s and contractions Stimulation 18 V 0 5 ms duration 2 Hz *a* control *b* noradrenaline 5×10^{6} g/ml (for 2 min) *c* noradrenaline 1×10^{6} g/ml (for 2 min *d* phentolamine 1×10^{6} g/ml (for 5 min) *e* phentolamine 1×10^{6} g/ml + noradrenaline 5×10^{6} g/ml (for 2 min) *f* control period 0 min after washing phentolamine Note the slight depolarization caused by phentolamine

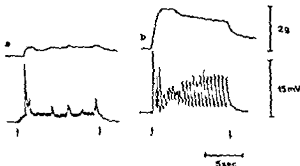


Fig 3 Sucrose gap recordings from a preparation stimulated repetetively over the period between arrows 10 V 0 5 ms duration 7 Hz *a* control record *b* record in the presence of noradrenaline 1×10^{-6} g/ml (for 2 min)

EJP s was still reduced by A or NA although considerably higher concentration of the catecholamines had to be used in order to demonstrate this effect clearly (Fig 4 c and d) Because the *a* blocker in high concentrations diminished the EJP.s by itself and depolarized the tissue (see below and Fig 1 d) it was not possible to establish whether this depressant effect on the EJP.s by the catecholamines could be completely abolished by phentolamine When these preparations were stimulated

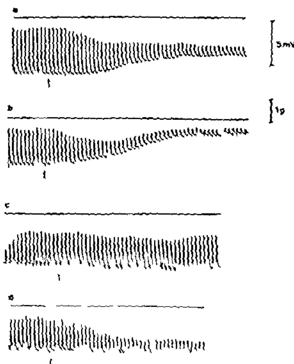


Fig 4 Sucrose gap recordings of EJP s from a guinea pig vas deferens. Stimulation 20 V 0 5 ms duration 0 4 Hz *a* at arrow adrenaline 5×10^{-8} *g/ml* *b* in the presence of propranolol 2×10^{-6} g/ml (for 5 *min*) at arrow adrenaline 5×10^{-8} g/ml *c* in the presence of propranolol 2×10^{-6} g/ml (for 20 *min*) and phentolamine 1×10^{-8} g/ml (for 15 min) at arrow adrenaline 5×10^{-8} g/ml *d* in the presence of propranolol 2×10^{-6} g/ml (for 40 min) and phentolamine 1×10^{-6} g/ml (for 35 *min*) at arrow adrenaline 5×10^{-6} g/ml



Fig 5 Sucrose gap recordings showing summating EJP s AP and contraction Stimulation 20 V 0 5 ms duration and 2 Hz *a* control record *b* nor adrenaline 5×10^{7} g/ml (for 2 min) *c* control record (5 min after washing *d*—*f* in the presence of propranolol 1×10^{6} g/ml (for 10 min) *d* control *e* noradrenaline 5×10^{7} g/ml (for 2 min) *f* control (5 min after washing noradrenaline) *g*—*i* in the presence of propranolol 1×10^{6} g/ml (for 30 min) and phentolamine 1×10^{6} g/ml (for 10 min) *g* control *h* noradrenaline 2×10 g/ml (for 2 min) *i* control (5 min after washing noradrenaline) Note reduction of EJP s and therefore delayed firing by propranolol and splitting of AP s by phentolamine

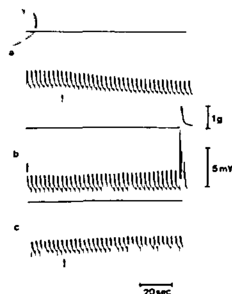


Fig 6 Sucrose gap recordings showing EJP s Stimulation 20 V 0 5 ms duration 0 4 Hz *a* at arrow isoprenaline 1×10^{6} g/ml producing EJP reduction and hyperpolarization *b* continuous recording from *a* showing effect of washing (at arrow) Note spiking during recovery from isoprenaline *c* in the presence of propranolol 1×10^{6} g/ml (for 5 min) at arrow isoprenaline 1×10^{6} g/ml

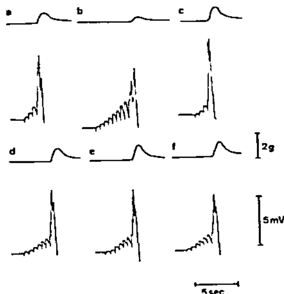


Fig 7 Sucrose gap recordings showing summating EJP s AP s and contractions Stimulation 18 V 0 5 ms duration 2 Hz *a* control *b* isoprenaline 1×10^{-6} g/ml (for 2 min) *c* 10 min after isoprenaline *d—f* in the presence of propranolol 1×10^{-6} g/ml (for 10 min) *d* control *e* isoprenaline 1×10^{-6} g/ml (for 2 min) *f* 10 min after isoprenaline

with frequencies of 2—3 Hz inducing summation of EJP s and a spike the inhibitory effect on the EJP s by catecholamines was further illustrated More EJP s were needed to elicit the spike despite the depolarization produced by the catecholamines (Fig 5 b) but the compound action potential once elicited was larger and the contraction became larger (Fig 5 b) The effect of noradrenaline on the AP and on contraction was not diminished but often enhanced by propranolol (Fig 5 d—f) On the other hand in the presence of phentolamine 1×10^{-6} g/ml the catecholamines could suppress spiking (Fig 5 h)

Effects of isoprenaline

In 6 out of 17 preparations isoprenaline 1×10^{-8}—1×10^{-6} g/ml had no detectable effect Larger concentrations (5×10^{-6}—1×10^{-5} g/ml) usually produced a slight depolarization However in 11 preparations a distinct hyperpolarization was seen (Fig 6 a) In these preparations isoprenaline also decreased the size of the EJP s After changing to Krebs solution containing no isoprenaline the membrane potential slowly returned to the initial level but before this was reached the EJP s increased in size and spiking often occured (Fig 6 b) The effect of isoprenaline on the EJP s and the membrane potential was further illustrated when stimuli were applied giving summating EJP s and eventually a spike (Fig 7) Then more EJP s were needed before the threshold was reached (Fig 7 b) Isoprenaline also reduced the spike and diminished the contraction (Fig 7 b) The effects of isoprenaline on the EJP s (Fig 6) and on the spike (Fig 7) were antagonized by propranolol 1—5×10^{-6} g/ml but not by phentolamine 1—5×10^{-6} g/ml

Effects of phentolamine and propranolol

The α and β blockers used for the analysis of the effects of catecholamines had

distinct effects by themselves Phentolamine 1—5×10⁻⁶ g/ml generally produced a slight depolarization of the vas deferens This was more marked with larger concentrations (5×10⁻⁶—1×10⁻⁵ g/ml (Fig 1 d and 2 d) In the concentration of 1×10⁻⁶ g/ml phentolamine had usually no overt effect on the EJP s but they were reduced by larger concentrations (1—5×10⁻⁵ g/ml) and abolished by 1×10⁻⁴ g/ml (This agree with the findings of Kuriyama (1963) and Burnstock and Holman (1964) In most of the preparations were phentolamine had depolarized the tissue the spikes increased in size and the contractions became slightly larger (Fig 2 d) In other preparations phentolamine caused a deterioration of the spike pattern (Fig 5 g and i) so that the spikes became more split but the contractions were still increased Prolonged exposure to phentolamine (15—30 min) also induced instability of the membrane potential and even spontaneous activity in the vas deferens

The most marked effect of *propranolol* 1×10⁻⁶ g/ml was that the drug decreased the size of the EJP s (Fig 4 b 5 d f and 7 d f) This would agree with the results of Mylecharane and Raper (1970) indicating a prejunctional effect of propranolol Propranolol had no overt effect on the spikes but increased the motor response occasionally *(Fig 5 d f) and differed in this respect from the catecholamines* which increased both the spike and the tension Therefore possibly propranolol may have an action directly on the contractile processes

Discussion

The enhancement by noradrenaline and adrenaline of the motor response to nerve stimulation is probably of the same nature as that of other smooth muscle stimulants and mainly due to the depolarization of the smooth muscle cell membrane (see discussion by Sjostrand 1973) This effect is an *α* effect since it is blocked by phentolamine

The present findings confirm the existence of inhibitory *β* receptors in the vas deferens *(Holman and Jowett 1964 Large 1965 and later investigators)* The *β*-effect is a hyperpolarization of the tissue and also a reduction of the size of the EJP s indicating a change in membrane conductance or perhaps a decreased trans mitter output To judge from the present results the relative number of *β* receptors may vary among different vas deferens preparations

In a minority of the preparations an additional inhibitory action of noradrenaline and adrenaline on the EJP s was observed which was not counteracted by propranolol and which could also be demonstrated after the depolarization had been blocked by phentolamine Nevertheless it is quite possible that this inhibition by catecholamines is an *α*-effect since larger concentrations of the catecholamines had to be used in order to demonstrate the reduction of the EJP s in the presence of phentolamine *Thus inhibitory action of the catecholamines is probably the basis of the non β* adrenergic inhibition by catecholamines on the motor response of the vas deferens to nerve stimulation observed by Hotta (1969) and by Ambache and Zar (1971) Since a bulk of biochemical morphological physiological and pharmacological evidence has been collected which strongly indicates that the main motor innervation

of the guinea pig vas deferens is adrenergic (*cf* Sjostrand 1965 Burnstock 1970 Holman 1970 and Swedin 1971) it seems reasonable to look for other possible explanations of the inhibitory action of the catecholamines on the EJP s than simply taking it as a proof of a nonadrenergic motor innervation of the vas deferens as claimed by Ambache and Zar (1971) The inhibitory action of the catecholamines on the EJP s will therefore be discussed on the assumption that the motor innervation of the vas deferens is mainly adrenergic

The inhibitory effect of the catecholamines on the motor transmission could be due to either an effect on the postjunctional membrane presumably a desensitization of junctional receptors or a junctional effect leading to a diminished release of the transmitter

The first explanation has been proposed by Hotta (1969) who suggests 2 different types of *a* adrenergic receptors junctional and non junctional receptors There is however no morphological evidence for a specialization of the smooth muscle cell membrane in junctional regions (*cf* Burnstock 1970) Furthermore when extra junctional receptors had been blocked by an *a* blocker in a concentration not affect ing junctional receptors if the catecholamines produced a desensitization of the junctional receptors one should have been able to observe at least a sign of a depolarization preceding the desensitizating as in other systems (*cf* Thesleff 1955 Katz and Thesleff 1957) This was never seen in my experiments Therefore this possibility seems unlikely

The second explanation an inhibition of the transmitter release appears to be the one to be favoured Thus in his thorough study Starke (1962) has presented strong evidence for an inhibition of adrenergic transmitter release from the rabbit heart caused by low concentrations of *a* receptor stimulants In addition catecholamines may also depress the transmitter output indirectly via *e g* release of prostaglandins (Gilmore Vane and Wyllie 1968) which can inhibit adrenergic transmitter release (*cf* Hedqvist 1970 Wennmalm 1971) Prostaglandins can be released from the vas deferens (Swedin 1971) and they suppress the motor response of the vas deferens to nerve stimulation (Euler and Hedqvist 1969 Ambache and Zar 1971) as well as the EJP s (Sjostrand 1972) Catecholamine induced release of prostaglandins appears further to be mediated by *a* receptors (Gilmore *et al* 1968)

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References

AMBACHE N and M A ZAR Evidence against adrenergic motor transmission in the guinea p g vas deferens *J Physiol* (Lond) 1971 *216* 359—389

BULBRING E and G BURNSTOCK Membrane potential changes associated with tachyphylaxis and potentiation of the response to stimulating drugs in smooth muscle *Brit J Pharmac* 1960 *15* 611—624

BURNSTOCK G Structure of smooth muscle and its innervation In *Smooth Muscle* Eds E Bulbring A Brading A Jones and T Tomita Arnold Publ London 1970 1—69

BURNSTOCK G and M E HOLMAN The transmission of excitation from autonomic nerve to smooth muscle *J Physiol* (Lond) 1961 *155* 115—133

BURNSTOCK G and M E HOLMAN An electrophysiological investigation of the actions of some autonomic blocking drugs on transmission in the guinea pig vas deferens *Brit J Pharmacol* 1964 *23* 600—612

BURNSTOCK G M E HOLMAN and H KURIYAMA Facilitation of transmission from autonomic nerve to smooth muscle of guinea pig vas deferens *J Physiol* (Lond) 1964 *17?* 31—49

BURNSTOCK G and R W STRAUB A method for studying the effects of ions and drugs on the resting and action potentials in smooth muscle with external electrodes *J Physiol* (Lond) 1958 *140* 156—167

EULER U S v and P HEDQVIST Inhibitory action of prostaglandins E_1 and E_2 on the neuromuscular transmission in the guinea pig vas deferens *Acta physiol scand* 1969 *77* 510—512

FURNESS J B and G BURNSTOCK A comparative study of spike potentials in response to nerve stimulation of the vas deferens of the mouse rat and guinea pig *Comp Biochem Physiol* 1969 *31* 337—345

GILMORE N J R VANE and J H WYLLIE Prostaglandins released by the spleen *Nature* (Lond) 1968 *218* 1135—1140

HEDQVIST P Studies on the effect of prostaglandins E_1 and E_2 on the sympathetic neuromuscular transmission in some animal tissue *Acta physiol scand* 1970 *79* Suppl 345

HOLMAN M Junction potentials in smooth muscle In *Smooth Muscle* Eds E Bulbring A Brading A Jones and T Tomita Arnold Publ London 1970 244—?88

HOLMAN M E and A JOWETT Some actions of catecholamines on the smooth muscle of the guinea pig vas deferens *Austr J exp Biol med Sci* 1964 *42* 40—53

HOTTA Y Some properties of the junctional and extrajunctional receptors in the vas deferens of the guinea pig *Agents and actions* 1969 *1* No 2 13—21

KATZ B and S THESLEFF On the factors which determine the amplitude of the miniature end plate potential *J Physiol* (Lond) 1957 *137* 267—278

KURIYAMA H Electrophysiological observations on the motor innervation of the smooth muscle cells in the guinea pig vas deferens *J Physiol* (Lond) 1963 *169* 213—228

LARGE B J Sympathetic β receptors and the guinea pig vas deferens *Brit J Pharmacol* 1965 24 194—?04

MYLECHARANE E J and C RAPER Prejunctional actions of some β adrenoceptor antagonists in the vas deferens preparation of the guinea pig *Brit J Pharmacol* 1970 *39* 128—138

SAXENA P R Effect of some drugs on the responses of the vas deferens and seminal vesicle to hypogastric nerve stimulation in guinea pig in vivo *Pharmacology* 1970 *3* 2?0—228

SJOSTRAND N O Effect of some smooth muscle stimulants on the motor response of the isolated guinea-pig vas deferens to hypogastric nerve stimulation *Nature* (Lond) 1961 *192* 1?—1191

SJOSTRAND N O The adrenergic innervation of the vas deferens and the accessory male genital glands *Acta physiol scand* 1965 *65* Suppl 257

SJOSTRAND N O A note on the dual effect of prostaglandin E_1 on the responses of the guinea pig vas deferens to nerve stimulation *Experientia* (Basel) 1972 *28* 431—43?

SJOSTRAND N O Effects of acetylcholine and some other smooth muscle stimulants on the electrical and mechanical responses of the guinea pig vas deferens to nerve stimulation *Acta physiol scand* 1973 *8?* 1—9

SJOSTRAND N O and C SWEDIN Potentiation by smooth muscle stimulants of the hypogastric nerve vas deferens preparation from normal and castrated guinea pigs *Acta physiol scand* 1968 *74* 47?—4?

STARKE K Alpha sympathomimetic inhibition of adrenergic and cholinergic transmission in the rabbit heart *Naunyn-Schmiedebergs Arch exp Path Pharmak* 197? ?74 18—45

SWEDIN C Studies on neurotransmission mechanisms in the rat and guinea pig vas deferens *Acta physiol scand* 1971 *83* Suppl 369

THESLEFF S The mode of neuromuscular block caused by acetylcholine nicotine decamethonium and succinylcholine *Acta physiol scand* 1955 *34* ?18—?31

WENNMALM A Studies on mechanisms controlling the secretion of neurotransmitters in the rabbit heart *Acta physiol scand* 1971 *82* Suppl 365

YAMAMOTO H and H NAKANISHI Effects of ganglionic blocking agents on responses of ductus deferens preparation of guinea pig to hypogastric nerve stimulation *Jap J Pharmacol* 1971 *14* ?56—?67

Acta physiol scand 1973 89 19—29
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Mucosal Hemodynamics in the Small Intestine of the Cat during Regional Sympathetic Vasoconstrictor Activation

By

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Abstract

SVANVIK J *Mucosal hemodynamics in the small intestine of the cat during regional sympathetic vasoconstrictor activation* Acta physiol scand 1973 89 19—29

The hemodynamics of the small intestinal mucosa of the cat was studied during electrical stimulation of the regional sympathetic vasoconstrictor nerves The indicator-dilution technique used allowed for a quantitative and separate investigation of the "mucosal as well as of the "villous intravascular flows and volumes After an initial marked mucosal vasoconstriction the villous intravascular plasma flow increased above control during prolonged sympathetic nervous activation in the face of a reduced intestinal venous outflow Concomitantly blood flow of the entire mucosal layer stabilized at about control Upon cessation of the nerve stimulation total venous outflow as well as mucosal blood flow showed an hyperemic response while "villous plasma flow remained unchanged The results thus suggest that blood flow in the intestinal villi is increased while flow in a more deeply situated region of the mucosa is decreased during regional sympathetic vasoconstrictor activation.

The effects of an activation of the regional sympathetic vasoconstrictor fibres to the intestinal vascular bed were studied in detail by Folkow and coworkers (Folkow *et al* 1964 a b Cobbold *et al* 1964 Dresel *et al* 1966 Dresel and Wallentin 1966 Wallentin 1967) It was shown that a continuous graded activation of the splanchnic nerves stimulation of the hypothalamic defence area or a continuous i a infusion of noradrenaline produce a characteristic response in this vascular bed Initially the intestinal blood flow is decreased largely in proportion to the stimulation frequency as a result of a constriction of the resistance vessels Concomitantly the capacitance vessels constrict as indicated by an abrupt decrease of tissue volume However within 2—4 min after the onset of constrictor fibre stimulation or noradrenaline infusion intestinal blood flow again increases (autoregulatory escape from vasoconstrictor fibre influence) reaching a new steady state level only moderately below control while the neurogenic capacitance response remains largely unaltered throughout the stimulation period During this steady state phase of vasoconstriction flow resistance seldom increases more than 100 per cent above control even at the highest physiological stimulation rates while the capillary filtration coefficient reflecting the surface available for transcapillary exchange is more drastically reduced (Folkow *e*

1964 b) Even though blood flow was usually only slightly reduced during the steady state phase an often pronounced reactive hyperemia was regularly seen upon cessation of the constrictor fibre stimulation

Using the rather crude method of India ink injections Folkow *et al* (1964 b) obtained evidence which together with the characteristic blood flow pattern, seemed to suggest a redistribution of blood flow within the intestinal wall upon nervous vasoconstriction thought to occur mainly from the mucosa towards the submucosa Experiments with the ^{86}Rb clearance technique suggested that the proposed neurogenic redistribution of flow did not involve any true shunting of blood but still took place along exchange vessels (Dresel Folkow and Wallentin 1966) The mentioned redistribution hypothesis has, however been challenged by other investigators (*e g* Baker and Mendel 1967 Ross 1971) Thus Ross (1971) using an indicator fractionation method failed to obtain any evidence of a decreased mucosal and increased submucosal blood flow at least during noradrenaline infusion

From the discussion above it is apparent that the adrenergic influence on the different parallel coupled vascular circuits in the intestinal wall is somewhat controversial calling for further investigation A method was recently developed in this laboratory using nondiffusible tracers (*e g* labelled red cells or plasma particles) which makes it possible to study separately and quantitatively the vascular reactions within the mucosa of the cat small intestine (Biber *et al* 1973 a) It was therefore considered of interest to use this technique to study the mucosal hemodynamics during constrictor fibre activation Parts of the results have previously been published in a preliminary form (Biber *et al* 1969 Haglund *et al* 1972)

Methods

The experiments were performed on ?2 cats under chloralose anesthesia (50—70 mg/kg b w) rived of food for at least 24 h and without signs of intestinal infection The theoretical kground and the operative procedures were essentially the same as described in an earlier per Biber *et al* 19 3 a) which also gives a full details regarding calculations of mucosal and l us plasma and red cell flows and volumes

To summarize briefly the method involves a continuous registration of venous outflow from an intestinal segment *in situ* by means of an optical drop recorder unit Concomitantly the transit of i a injected labelled red cells (^{32}P or plasma colloids (^{32}P or ^{198}Au) was monitored by one detector (Geiger Muller tube or semiconductor tube) placed in the lumen of the gut When using ^{32}P the monitored tissue region includes most of the mucosa while only the villi are "seen" with ^{198}Au Knowing the total tracer amount injected and total intestinal blood flow mucosal plasma or red cell flows can be estimated from the height of the recorded indicator dilution curves Biber *et al* 1973 a Furthermore regional plasma or red cell content can be determined from the areas under the registered curve

The nerves surrounding the superior mesenteric artery were cut and the peripheral ends were mounted on a bipolar silver electrode Nerve stimulation was performed with square wave pulses (10 V 5 ms and 8 Hz Atropine 1—1 5 mg/kg b w was given i v to block the concomitantly activated vagal cholinergic fibres to the intestine

Results

A ^{198}*Au labelled colloid plasma particles*

^{198}Au labelled plasma particles were monitored at their passage through the hairpin vascular loops of the villi Biber *et al* 1973 a) Parameters measured with this tracer are therefore designed villous

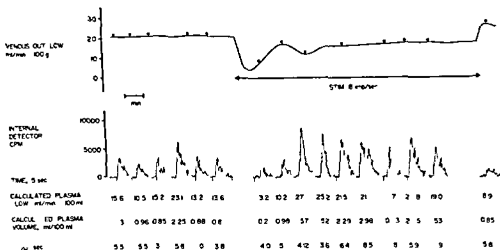


Fig 1 Cat 2 7 kg b w arterial hematocrit 30 % The effect of stimulation (8 Hz 10 V 5 ms) of the regional sympathetic vasoconstrictor fibres on the blood flow in the mesenteric vein (upper panel) and on "villous plasma flow volume and mean transit time (t_{VH}) These parameters were calculated from the indicator dilution curves shown in middle part of the figure registered after i a. injections of 25 μl of ^{195}Au labelled colloid particles. Time of injection is indicated in upper curve by dot For details see text.

In Fig 1 a typical experiment is illustrated where the periarterial nerves were stimulated at 8 Hz for 13 min Total intestinal blood flow as measured in the mesenteric vein is shown in the upper panel The transit of repeated i a injections of 25 μl ^{195}Au labelled colloid plasma particles measured with an intraluminal semiconductor detector can be followed from the indicator-dilution curves shown From these curves villous plasma flow plasma volume and mean transit time (t_{VH}) can be calculated (Biber *et al* 1973 a) as shown in the table below the curves The venous outflow recording illustrates the characteristic initial vasoconstriction upon constrictor fibre activation *followed by the autoregulatory escape from vasoconstrictor fibre influence* earlier discussed The calculated villous plasma flow and volume became markedly reduced during the initial vasoconstriction In some experiments the initial vasoconstriction of the villous vessels was so pronounced that no tracer was recorded by the semiconductor detector during this phase However *during the steady state phase of vasoconstriction villous flow and volume were* somewhat larger than control values despite a significantly reduced total intestinal blood flow t_{VH} stayed fairly constant throghout the experiment of Fig 1 Upon cessation of stimulation no hyperemia was seen in the villi while total venous out flow was increased above prestimulatory control

The cumulated data from 10 cats on the effects of vasoconstrictor fibre activation at 8 Hz on villous plasma flow plasma volume and t_{VH} are shown in Fig 2 The results illustrated in this Fig are similar to those of Fig 1 A pairing design test shows that villous plasma flow as well as villous plasma volume were significantly higher ($p < 0.05$) than control during the steady state phase of vasoconstriction —

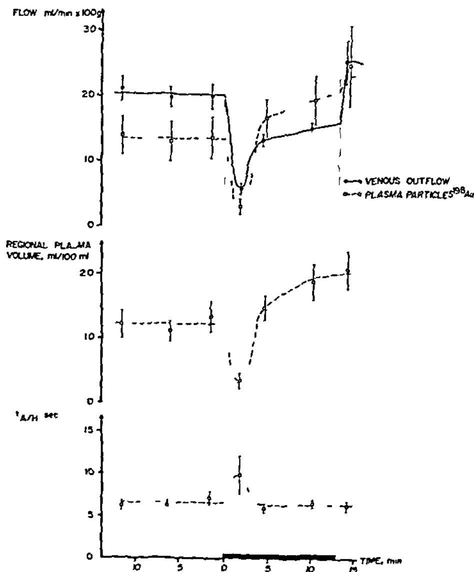


Fig 2 Cumulated data from 10 cat experiments on the effects of vasoconstrictor fibre activation at 8 Hz on the "villous" hemodynamics The upper panel illustrates total venous outflow expressed in ml/min × 100 g intestinal tissue and "villous" plasma flow in ml/min × 100 ml "villous" tissue Changes in "villous" plasma volume and mean transit time ($\bar{t}_{A/H}$) are shown in the middle and lower panels respectively Each cat is represented by only one value in each point Mean arterial hematocrit was 34.1 ± 2.2 (S E) per cent Lines drawn by inspection Bars indicate ± S E

Thus Fig 1 and 2 clearly show that villous plasma flow is increased in the face of a reduced total intestinal blood flow during steady state constrictor fibre influence suggesting that a larger portion of total blood flow is diverted to the villi Assuming a certain length of the capillarized part of the villus it was possible to calculate from the present data the fraction of intestinal plasma flow passing villi Fig 3 shows the

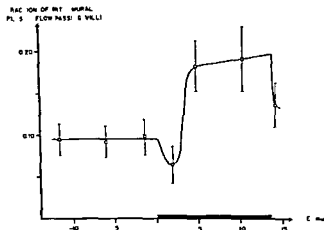


Fig 3 Fraction of the intramural plasma flow passing the villous capillaries calculated from the villous plasma flow values shown in Fig 2 and assuming that the length of the capillarized part of the villi was 0 7 mm Lines drawn by inspection Bars indicate ±S E Note the comparatively low "resting" value indicating a high "resting vascular tone of the villous vascular bed in this series of experiments

results of such calculations assuming a villous length of 0 7 mm. It is clear from this figure that after an initial decrease a larger fraction of total plasma flow was distributed to the villi during nervous vasoconstriction than during control

B ^{32}P labelled red cells and plasma particles

^{32}P labelled blood particles will with the technique used be monitored at their passage through the entire mucosa (Biber *et al* 1973 a) Parameters measured with this tracer is therefore designed mucosal The results reported below are based on 12 cat expts in which ^{32}P labelled red cells and plasma particles were injected during rest and during sympathetic stimulation In 6 of these expts two periods of stimulation were performed and red cells were injected during one period and plasma particles during the other In the other 6 expts labelled red cells and plasma particles were alternatingly injected during the same resting and stimulation periods

Fig 4 illustrates the changes in mucosal red cells flow (upper panel) and volume (lower panel) as well as mucosal plasma flow and volume as induced by vasoconstrictor fibre stimulation The typical response pattern of the total intestinal blood flow is seen in the upper curve of the upper panel All mucosal parameters studied showed a decline concomitant to the initial marked neurogenic vasoconstriction Mucosal red cell flow and volume then returned to control values while plasma flow and volume tended to increase above control during the steady state phase of vasoconstriction A pairing design test failed however to reveal any significantly higher mucosal plasma flow during the steady state phase as compared to control ($p > 0.30$) When the stimulation period ended mucosal red cell and plasma flows were transiently increased further above prestimulatory control (*cf* Fig 2)

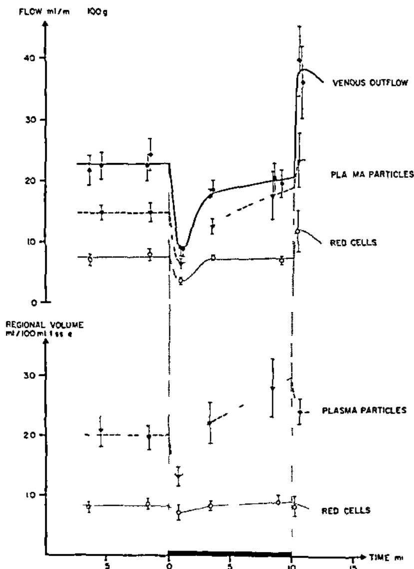


Fig 4 Cumulated data from 12 cat expts on the effects of vasoconstrictor fibre activation at 8 Hz on the "mucosal hemodynamics as measured with ^{32}P labelled red cells and plasma particles The upper panel shows the venous outflow expressed as ml/min × 100 g intestinal tissue and regional plasma and red cell flow as ml/min × 100 ml "mucosal tissue Lower panel shows the regional content of plasma and red cells in ml/100 ml mucosal tis ue Each cat is represented by only one value in each point Mean arterial hematocrit was 33 9 ± 1 1 (S E) per cent Lines drawn by inspection bars indicate ± S E

In 11 expts mucosal red cell volume was determined with the equilibration method (total volume see Biber *et al* 1973 a) and from the area under the registered indicator-dilution curve (perfused volume) The accumulated data from these expts are shown in Fig 5 indicating that the mucosal total red cell

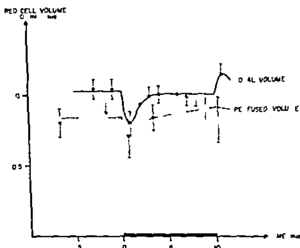


Fig 5 Cumulated data from 11 cat expts in which mucosal red cell volume was determined with both the method using slug injections ("perfused" red cell volume) and the method of tracer equilibration in the circulating blood ("total" red cell volume) during sympathetic activation Each cat is represented only once in each point Mean arterial hematocrit was 31 5±1 2 (S E) per cent Lines drawn by inspection bars indicate ±S E

volume was somewhat larger than the "perfused" volume but showed a similar response to sympathetic stimulation However an increased "total" volume above control was seen upon cessation of stimulation while the "perfused" red cell volume remained unchanged

The effects of sympathetic stimulation on the mean transit time for red cells and plasma through the mucosa are shown in Fig 6 The plasma $\bar{t}_{A/H}$ was consistently higher than that for red cells as expected from the laminar flow of blood

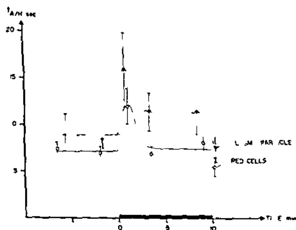


Fig 6 Cumulated data from the expts of Fig 5 on the effect of nervous vasoconstriction on mean transit time ($\bar{t}_{A,H}$) for ^{32}P labelled red cells and plasma particles through the mucosal vessels Each cat is represented only once in each point Lines drawn by inspection bars indicate ±S E

Discussion

The present investigation on the vascular bed of the small intestine was performed in order to analyze in detail the mucosal hemodynamics during stimulation of the regional sympathetic vasoconstrictor fibres. To this end the splanchnic nerves were stimulated at 8 Hz, a stimulation frequency at the upper end of the "physiological" range, and the vascular reactions in the mucosal compartments of the intestinal wall were studied by an indicator dilution technique.

The initial pronounced decrease of total intestinal blood flow was accompanied by a marked decrease of blood flow and blood content of the "villous" as well as of the "mucosal" vessels (Fig. 2 and 4). As intestinal blood flow again increased as a result of the "autoregulatory escape" from vasoconstrictor fibre influence, villous plasma flow was augmented to values significantly above control while total intestinal blood flow remained somewhat below control. "Mucosal" red cell and plasma flows, on the other hand, did not exceed prestimulatory control values. When combined, these observations suggest that the steady state phase of vasoconstrictor fibre influence on the vasculature of the intestinal mucosa is differentiated, allowing for an increased flow through the villous hairpin loops while the flow to more deeply situated regions, possibly around the crypts, evidently remains reduced. This becomes clear if one subtracts the "villous" plasma flow values from those of the total mucosa in the steady state phase of nervous vasoconstriction. This conclusion is also supported by the observation that the "villous" vessels did not exhibit any poststimulatory hyperemia while the "mucosal" blood flow increased upon cessation of nervous stimulation.

The present data on the neurogenic changes in mucosal blood flow are thus con... with the views of Folkow *et al.* (1964 b) in so far as a redistribution of blood occurs in the intestinal wall upon sympathetic stimulation. However, the direc...n of this redistribution is according to the present results opposite to the one proposed in these earlier studies, i.e. flow seems to be directed from the deeper to the more superficial layers of the intestinal mucosa. The conclusions reached in the present study are corroborated by the findings that the absorption of ^{85}Kr from the lumen via the villous vessels is not altered during sympathetic vasoconstriction (Lundgren and Svanvik 1968, Svanvik, to be published).

In this connection it should be pointed out, however, that the radioactivity in the submucosal layer is recorded with a comparatively low efficiency (Biber *et al.* 1973 a) and vascular reactions in this wall layer can hence not be followed with the present method. Since observations made with the inert gas wash-out technique (Lundgren, unpublished observatins) suggest that the intestinal muscularis blood flow is somewhat decreased during the steady state phase of nervous stimulation in the face of a largely unchanged "mucosal" blood flow (Fig. 4), one cannot entirely exclude the possibility that blood flow is increased in the submucosa.

The neurogenic changes in regional intravascular volumes were qualitatively similar to the flow alterations described above, supporting the conclusions above regarding a differentiated sympathetic control of the mucosal vessels. The changes

of *total* intestinal blood volume caused by nerve stimulation differs, however markedly from the results illustrated in Fig 2 and 4 Upon stimulating the splanchnic nerves at 8 Hz about 40 per cent of the intestinal blood volume i e about 3 ml/100 g tissue is expelled from the gut (Folkow *et al* 1964 b) Furthermore the constriction of the intestinal capacitance vessels is well maintained throughout the stimulation period at least when venous outflow pressure is kept above 10 cm H_2O so that the component due to passive elastic changes is largely eliminated This difference in results is probably explained by the fact that most of the intestinal intravascular blood volume is contained in the large mesenteric veins and venules situated mainly in the mesentery in the muscularis and in the submucosa (*cf* Jodal and Lundgren 1970) Thus detectors registering radioactivity mainly from the mucosa will only monitor smaller part of the capacitance section of the intestinal wall It is further possible that the neurogenic influence is more pronounced on the larger veins and venules which were not seen by intraluminal detectors

Changes in the intravascular volume of the intestinal mucosa particularly in the villi, not only reflect alterations in regional red cell and plasma content but also indirectly the capillary surface area (*cf* Biber *et al* 1973 b) This is especially true for the villous plasma volume as measured in the present study since the villi consist almost exclusively of capillaries The mucosal plasma content also gives a fairly good indirect measure of villous capillary surface area, since hematocrit of the villous blood is only around 50 per cent of arterial Thus when measuring ^{32}P labelled plasma colloids by an intraluminal Geiger Muller tube a large amount of the tracer is situated in the vessels most adjacent to the detector Similarly it is reasonable to assume that the mucosal red cell volume is mainly confined to the deeper parts of the mucosa

The recorded alteration of the villous and mucosal plasma volumes suggest that the perfused capillary surface area in the villi is if anything somewhat increased during the steady state phase of neurogenic intestinal vasoconstriction Folkow *et al* (1964 b) recorded the alterations in the total capillary surface area in the cat small intestine by determining the capillary filtration coefficient (CFC) This parameter was markedly reduced during nervous vasoconstriction often below half the control values suggesting a pronounced closure of precapillary sphincters probably of mucosal vessels since it seems reasonable to assume that the fenestrated mucosal capillaries have a higher porosity than the continuous muscularis capillaries and will therefore contribute decidedly more to the measured intestinal CFC Hence the present observation of an increased villous capillary surface area during neurogenic vasoconstriction in the face of a markedly reduced intestinal CFC probably implies that the precapillary sphincters of some other richly capillarized mucosal region are effectively closed during sympathetic influence

To summarize the present study seems to indicate that after an initially pronounced neurogenic constriction of all the mucosal vessels the vessels supplying the villi dilate and villous flow as well as blood volume then becomes increased above prestimulatory control Vasoconstriction is however maintained in a more deeply situ

Discussion

The present investigation on the vascular bed of the small intestine was performed in order to analyze in detail the mucosal hemodynamics during stimulation of the regional sympathetic vasoconstrictor fibres To this end the splanchnic nerves were stimulated at 8 Hz a stimulation frequency at the upper end of the physiological range and the vascular reactions in the mucosal compartments of the intestinal wall were studied by an indicator dilution technique

The initial pronounced decrease of total intestinal blood flow was accompanied by a marked decrease of blood flow and blood content of the villous as well as of the mucosal vessels (Fig 2 and 4) As intestinal blood flow again increased as a result of the autoregulatory escape from vasoconstrictor fibre influence villous plasma flow was augmented to values significantly above control while total intestinal blood flow remained somewhat below control Mucosal red cell and plasma flows on the other hand did not exceed prestimulatory control values When combined these observations suggest that the steady state phase of vasoconstrictor fibre influence on the vasculature of the intestinal mucosa is differentiated allowing for an increased flow through the villous hairpin loops while the flow to more deeply situated regions possibly around the crypts evidently remains reduced This becomes clear if one subtracts the villous plasma flow values from those of the total mucosa in the steady state phase of nervous vasoconstriction This conclusion is also supported by the observation that the villous vessels did not exhibit any poststimulatory hyperemia while the mucosal blood flow increased upon cessation of nervous stimulation

The present data on the neurogenic changes in mucosal blood flow are thus consistent with the views of Folkow *et al* (1964 b) in so far as a redistribution of blood occurs in the intestinal wall upon sympathetic stimulation However the direction of this redistribution is according to the present results opposite to the one proposed in these earlier studies *i e* flow seems to be directed from the deeper to the more superficial layers of the intestinal mucosa The conclusions reached in the present study are corroborated by the findings that the absorption of ^{85}Kr from the lumen via the villous vessels is not altered during sympathetic vasoconstriction (Lundgren and Svanvik 1968 Svanvik to be published)

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The neurogenic changes in regional intravascular volumes were qualitatively similar to the flow alterations described above supporting the conclusions above regarding a differentiated sympathetic control of the mucosal vessels The changes

FOLKOW B D H LEWIS O LUNDGREN S MELLANDER and I WALLENTIN The effect of graded vasoconstrictor fibre stimulation on the intestinal resistance and capacitance vessels *Acta physiol scand* 1964 a *61* 445—457

FOLKOW B D H LEWIS O LUNDGREN S MELLANDER and I WALLENTIN The effect of the sympathetic vasoconstrictor fibres on the distribution of capillary blood flow in the intestine *Acta physiol scand* 1964 b *61* 458—466

FOLKOW B O LUNDGREN and I WALLENTIN Studies on the relationship between flow resistance capillary filtration coefficient and regional blood volume in the intestine of the cat *Acta physiol scand* 1963 *57* 270—283

HAGLUND U and O LUNDGREN The effects of vasoconstrictor fibre stimulation on consecutive vascular sections of cat small intestine during hemorrhagic hypotension *Acta physiol scand* 1973 *88* 95—108

HAGLUND U O LUNDGREN and J SVANVIK On the pathogenesis of the intestinal mucosal lesions in shock *Acta physiol scand* 1973 *87* 49 A

JODAL M and O LUNDGREN Regional distribution of red cells plasma and blood volume in the intestinal wall of the cat *Acta physiol scand* 1970 *80* 533—537

LILLEHEI R C R H DIETZMAN and S MOVAS The visceral circulation in shock *Gastroenterology* 1967 *52* 468—471

LUNDGREN O and J SVANVIK Uptake of ^{85}Kr from the lumen of the small intestine to the intestinal blood in the cat *Acta physiol scand* 1968 *74* 20A—21A

LUNDGREN O and J SVANVIK Mucosal hemodynamics in the small intestine of the cat during reduced perfusion pressure *Acta physiol scand* 1973 *88* 551—563

ROSS G Effects of norepinephrine infusions on mesenteric arterial blood flow and its tissue distribution *Proc Soc exp Biol* (N Y) 1971 *137* 971—974

WALLENTIN I Studies on intestinal circulation *Acta physiol scand* 1967 *69* Suppl 279

Acta physiol scand 1973 89 30—38
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An Investigation of the Action of Diphenylhydantoin on Sodium Efflux in Barnacle Muscle Fibres

By

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Abstract

BITTAR E E S S CHEN, B G DANIELSON and E Y TONG *An investigation of the action of diphenylhydantoin on sodium efflux in barnacle muscle fibres* Acta physiol scand 1973 *89* 30—38

Sodium efflux from barnacle fibres is suppressed by external but not by internal application of diphenylhydantoin (DPH) DPH (10^{-4} M) at pH 7 8 reduces the Na efflux by 3[illegible] % a value slightly less than half that obtained with 10^{-4} M ouabain Ouabain has the ability to abolish the remaining Na efflux whereas DPH incompletely abolishes the ouabain insensitive Na efflux The onset of inhibition by DPH is slower than that by ouabain At lower external pH the onset of peak action by DPH is more rapid than at pH 7 8 The sensitivity of the Na efflux to DPH is unaffected by external K However the residual efflux from fibers poisoned with ouabain following K removal is insensitive to DPH DPH prevents the Na efflux from responding to external acidification It also has the power to almost completely reverse the effect of ternal acidification on the Na efflux It is provisionally concluded that the T system is the site PH action

the preceding paper concerned with the inhibitory action of ouabain (Bittar *et al* 1973) the view was advanced that the Na pump of barnacle muscle fibers includes an ouabain insensitive component which is stimulated by low external pH as well as by microinjecting CaCl The object of the experiments here described was to obtain further chemical information about the nature of this component of the Na efflux mechanism by using the anti epileptic drug diphenylhydantoin (DPH) According to Pincus and Giarman (1967) Rawson and Pincus (1968) and Peter (1970) DPH has the ability to suppress *in vitro* the activity of the Na—K ATPase from rat and guinea pig brain and from skeletal muscle These observations are in sharp conflict with those of Woodbury (1955 1969) who reported that DPH stimulates the Na efflux both in brain and skeletal muscle It therefore seemed particularly desirable to find out whether DPH produces an effect on active Na transport in the barnacle fiber which as an experimental preparation offers the possibility for studies of such problems both more acurately and meaningfully than in tissue slices or cell free

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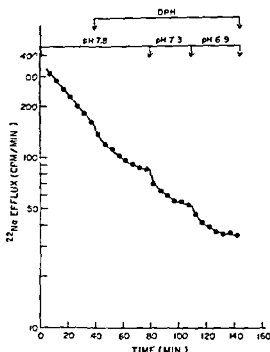


Fig 3 The effect on the Na efflux from a barnacle fiber of 10 ⁴ M DPH before and after reducing the external pH to 7 3 and 6 9

and the activity remaining in the fiber at the end of each experiment was basically as described by Bittar (1966) The collection of the effluent started about 15 min after the injection of the fibre During this period the equilibration between the injected ^{22}Na and the internal ^{23}Na occurs Bittar *et al* 1972) A well type counter (Panax model CoWP) fitted with a solid phosphor connected to a scaler manufactured by Nuclear Chicago was employed

Results

In the first groups of experiments the effect of DPH on the Na efflux was tested. As illustrated in Fig 1 10 ⁴ M DPH when applied externally caused an apreciable but slow fall in the Na efflux Full effect was not achieved until 40 min after application Estimates of the magnitude of the effect based on the change in rate constant for sodium efflux caused by a 10 ⁴ M concentration of DPH gave an average value of 32 % with a range of 20—56 % (53 expts)

The next group of experiments was designed to determine the maximal effective concentration of DPH Shown in Fig 2 is the result of an experiment in which a range of DPH concentrations was used In this case the inhibition caused by DPH on the Na efflux is plotted as a percentage against the log molar concentration used. As indicated greatest inhibition occurred when a concentration of 10 ⁴ M DPH was employed *The effect of higher concentrations could not be examined* on account of the low solubility of DPH at pH 7 8

Since the pKa value for DPH is 8 3 (Agarwal and Blake 1968) it was a matter of some interest to find out whether the external pH influences the onset of the full effect of DPH. A typical result recorded in Fig 3 indicates that lowering the external

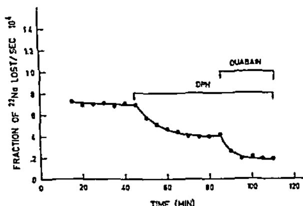


Fig 4 The effects of external application of 10^{-4} M DPH followed by 5×10^{-5} M ouabain on the loss of sodium from a barnacle fiber

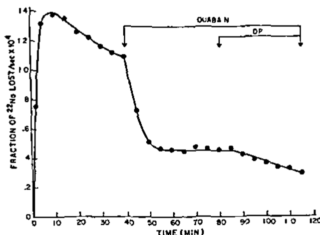


Fig 5 The effects of external application of 5×10^{-5} M ouabain followed by 10^{-4} M DPH on the efflux loss of sodium from a barnacle fiber

pH first to 7 3 and then 6 9 resulted in more rapid appearance of the peak effect of DPH (5 expts) Attempts at lowering further the external pH were avoided because stimulation of the Na efflux is known to follow whenever an external pH values of 6 8 is approached (Bittar and Tong 1971 Danielson *et al* 1971 b)

The finding that DPH slowly reduces the Na efflux raised the possibility that the point of action of this agent may not be the same as that of ouabain In order to shed some light on this question experiments were carried out which fall into two groups In the first group external application of DPH was followed by ouabain and in the second group ouabain was followed by DPH As illustrated in Fig 4 the effect of 10^{-4} M DPH developed rather slowly whereas that of 5×10^{-5} M ouabain developed more rapidly (11 expts) This suggested that DPH and ouabain may act at 2 different places in the plasma membrane *e g* the T system and the plasma membrane proper or that the action of DPH on the ouabain sensitive Na efflux located either in the plasma membrane or in the plasma membrane and T system is incomplete

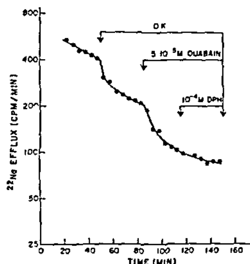


Fig 6 Lack of effect of 10 4 M DPH on the residual Na efflux from a barnacle fiber following K removal and poisoning of the fiber with 5×10 5 M ouabain

In the second group of experiments the procedure adopted was to apply ouabain followed by DPH A typical experiment is given in Fig 5 where it can be seen that 5×10 5 M ouabain caused a fairly sharp fall in the rate of Na loss leaving a small Na efflux which was incompletely abolished by 10 4 M DPH during the treatment period alowed (5 expts) From these results it remained unclear whether the action of ouabain was complete As found earlier a better way of inactivating the active transport system was by K removal followed by the addition of ouabain (Bittar *et al* 1973 c) Experiments were therefore designed to check whether DPH has the ability f abolishing the residual Na efflux As shown in Fig 6 both K removal and the lication of 5×10 5 M ouabain produced an appreciable reduction in the Na flux while 10 4 M DPH failed to interrupt the residual loss of Na (8 expts) It

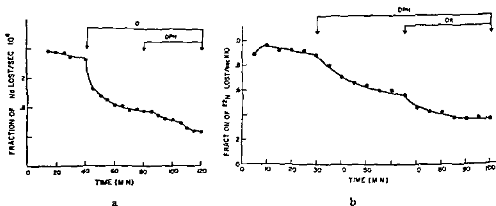


Fig 7 a. The effect on the fractional Na loss from a barnacle fiber of K removal followed by 10 4 M DPH

Fig 7 b The effect on the fractional Na loss from a barnacle fiber of 10 4 M DPH followed by K removal

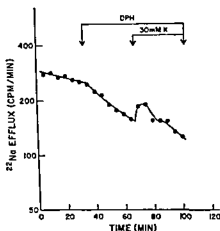


Fig 8

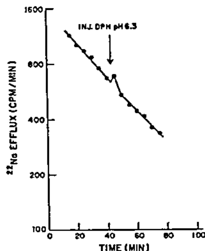


Fig 9

Fig 8 The effect on the fractional Na loss from a barnacle fiber of 10^{-4} M DPH followed by a 30 mM K solution

Fig 9 Lack of effect of internal application of a 1 cm column of a saturated solution of DPH at pH 6 3 on the sodium efflux from a barnacle fiber

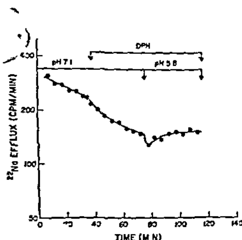
thus emerged that DPH and ouabain share a common site which is not dependent on external K Supporting evidence for this view was obtained from experiments in which the effect of DPH on Na efflux into a K free solution and the effect of K removal on the DPH insensitive Na efflux were tested (6 expts of each type) Typical results are presented in Fig 7 a and b These indicate that the residual Na efflux in either case is further reduced by DPH or K removal

One more way of substantiating the view that DPH at pH 7 8 inactivates a K independent moiety of the transport system was to compare the response to high K of fibres poisoned by ouabain with the response to high K of fibers poisoned by DPH The results of the 5 expts done show that raising the external K to 30 mM after applying 10^{-4} M DPH leads to only a small rise in the residual Na efflux This result shown in Fig 8 is in striking contrast to that reported by Danielson *et al* (1971 c) who found that the ouabain insensitive Na efflux is greatly stimulated by raising the external K to 30 mM It clearly implies that the DPH sensitive site is not a part of the ouabain insensitive Na efflux mechanism

In view of the slowness of DPH action it became necessary to determine whether or not the point of action of DPH was the inner side of the plasma membrane Hence experiments were done with a saturated solution of DPH ($> 10^{-4}$ M) As shown in Fig 9 internal application of a saturated solution of DPH at pH 6 3 was without effect on the Na efflux (3expts) A similar result was obtained with a solution at pH 7 8 (4 expts) This led to the microinjection of a more concentrated solution by employing a solvent composed of 40 % propylene glycol and 10 % alcohol in water Control experiments involving the injection of a 1 cm column of the

solvent at pH 12 (4 expts.) indicated no change in the behaviour of the Na efflux. Further internal application of a 50 mg/ml DPH solution at pH 12 was also ineffective (4 expts.). Adjustment of the pH of the solution to 7.0 prior to injection made no difference (4 expts.). Taken together, these results with injected DPH meant that the point of action of DH was the outer side of the plasma membrane.

Consideration was next given to the possibility that DPH could be used as a probe for the ouabain insensitive component following its activation by the external acidity. The result illustrated in Fig. 10a shows quite clearly that 10^{-4} M DPH almost completely prevented the Na efflux from responding to the addition of protons to the bathing medium (17 expts.). Thus, when 10^{-4} M DPH was applied externally there followed a gradual fall-off in the Na efflux, and when the external pH was lowered from 7.8 to 5.8 a small, transient fall in the residual Na efflux took place. This was followed by an only slight rise in the Na efflux. It should be remembered that as a rule barnacle fibers are very sensitive to external acidity. This fact is illustrated by Fig. 10b, where the response of a control fiber to an external pH of 5.8 is shown. That DPH behaves as a specific blocking agent of the ouabain insensitive pump component was further confirmed in the second group of experiments in which 10^{-4} M DPH was introduced into the bathing medium following stimulation of the Na efflux by reducing the external pH to 6.3 (11 expts.). As shown in Fig. 11, the addition of 10^{-4} M DPH produced an appreciable but slow fall in the Na efflux. Restoration of the external pH to 6.9 following the onset of the full effect of DPH is also shown to result in a further reduction of the residual loss of Na. This is in accord with the concept that DPH fails to completely inactivate the stimulated ouabain insensitive Na efflux.



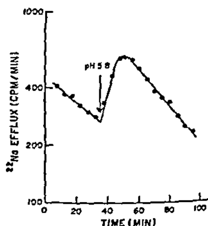


Fig. 10a. The behaviour of the sodium efflux from a barnacle fiber during external treatment with 10^{-4} M DPH at pH 7.1 and then at pH 5.8.

Fig. 10b. The effect of reducing the external pH to 5.8 on the sodium efflux from a barnacle fiber isolated from the same muscle bundle as that used in the preceding experiment.

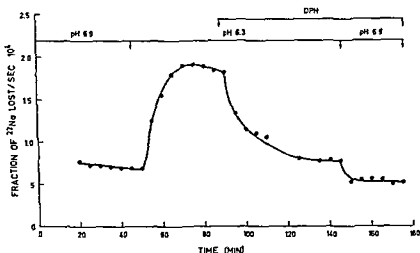


Fig 11 The effects on the sodium loss of reducing the external pH from 6.9 to 6 3 followed by 10 4 M DPH and then raising the external pH to 6 9

Discussion

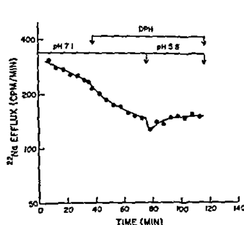
The experiments reported here make it at once clear that DPH is an inhibitor of the Na pump and that its site of action is a K independent moiety of the ouabain sensitive Na efflux mechanism Evidence of delay in the appearance of the peak action of DPH at pH 7 8 supports the argument that its site of action is the T system rather than the pla ma membrane proper That this site is located on the outer side of the T system is indicated by the failure of internally applied DPH to modify the behaviour of the Na efflux

The experiments carried out at a low external pH provided clear-cut evidence that DPH is a genuine blocking agent of the ouabain insensitive Na efflux stimulated by a low external pH That this second pump component does not behave in the same way as an Na K ATPase system is suggested by two observations first the failure of ouabain to reduce the magnitude of the Na efflux present following external acidification (Danielson *et al* 1971 c) and second the slow onset of peak action by DPH at low external pH Since DPH at pH 7 8 also exerts its effect rather slowly, it may with reason be argued that there exists a common site which is relatively insensitive to external K and which is inactivated by ouabain only in the ab ence of external K. If this is so the significance of this site in relation to the mechanism stimulated by external acidification has yet to be elucidated

It remains to be considered whether the present experiments have some practical bearing For example the plasma level achieved when DPH is employed as an anti convulsive is in the region of 4×10 M (Lund *et al* 1964) and 4×19 5—6 5×10 5 M when DPH is employed as an anti arrhythmic agent (Lang *et al* 1965) These

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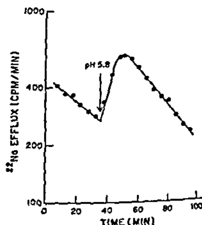


Fig 10 a. The behaviour of the sodium efflux from a barnacle fiber during external treatment with 10^{-4} M DPH at pH 7 1 and then at pH 5 8

Fig 10 b The effect of reducing the external pH to 5 8 on the sodium efflux from a barnacle fiber isolated from the same muscle bundle as that used in the preceding experiment

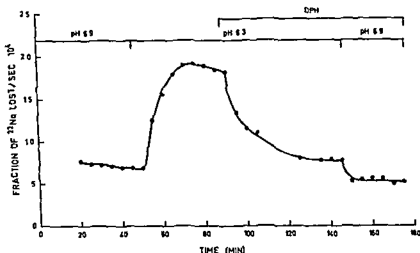


Fig 11 The effects on the sodium loss of reducing the external pH from 6 9 to 6 3 followed by 10^{-4} M DPH and then raising the external pH to 6 9

Discussion

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concentration ranges are not very different from that of 10^{-4} M used in the present work. It is also well to remember that the barnacle fiber has an elaborate T system (Selverston 1967). So does cardiac muscle (Nayler 1970). There are thus grounds for believing that further studies of the barnacle fibre may provide some clues as to the mechanism by which DPH acts as an anti-convulsant and anti arrhythmic agent.

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References

AGARWAL S P and M I BLAKE Determination of the pKa value for 5 5-Diphenylhydantoin *J pharm Sci* 1968 *57* 1434—1439

BITTAR E E Effect of inhibitors and uncouplers on the Na pump of the Maia muscle fiber *J Physiol* (Lond) 1966 *187* 81—103

BITTAR E E and E Y TONG Sensitivity of Na efflux from single barnacle muscle fibers to external H ions *Life Sci* 1971 *10* 43—48

BITTAR E E S CHEN B G DANIELSON H A HARTMANN and E Y TONG An investigation of sodium transport in barnacle muscle fibres by means of the micro-syringe technique *J Physiol* (Lond) 1972 *221* 389—414

BITTAR E E S CHEN B G DANIELSON and E Y TONG An investigation of the action of ouabain on the sodium efflux in barnacle muscle fibers *Acta physiol scand* 1973 *87* 37—390

CALDWELL P C and G E WALSTER Studies on the microinjection of various substances into crab muscle fibres *J Physiol* (Lond) 1963 *169* 353—372

DANIELSON B G E E BITTAR S CHEN and E Y TONG The effect of diphenylhydantoin on sodium efflux from single barnacle muscle fibers *Life Sci* 1971 a *10* 437—443

DANIELSON B G E E BITTAR S CHEN and E Y TONG Diphenylhydantoin as a blocking agent of the proton sensitive component of Na efflux in barnacle muscle fibers *Life Sci* 1971 b *13* 721—726

DANIELSON B G E E BITTAR S CHEN and E Y TONG The influence of low pH high K and microinjected $CaCl_2$ on the ouabain insensitive component of sodium efflux in barnacle muscle fibers *Life Sci* 1971 c *10* 833—839

HODGKIN A L and R D KEYNES Experiments on the injection of substances into squid giant axons by means of a microsyringe *J Physiol* (Lond) 1956 *131* 592—616

LANG T W H BERNSTEIN F F BARBIER H GOLD and E CORDAY The use of diphenyl hydantoin for the treatment of digitalis toxicity *Arch int Med* 1965 *116* 573—000

LUND M K S JORGENSEN and V KUHL Serum diphenylhydantoin in ambulant patients *Epilepsia* 1964 *5* 51—00

NAYLER N G Ion movements in heart muscle In *Membrane and Ion Transport* Vol 2 E E Bittar (Ed) Wiley Interscience London 1971 75—94

PETER J B A (Na + K)ATPase of sarcolemma from skeletal muscle *Biochem biophys Res Commun* 1970 *40* 1362—1369

PINCUS J H and N J GIARMAN The effect of diphenylhydantion of sodium potassium, magnesium activated adenosine triphosphatase in microsomal fractions of rat and guinea pig brain and on whole homogenates of human brain *Biochem Pharmacol* 1968 *17* 573—582.

RAWSON M D and J H PINCUS The effect of diphenylhydantoin on sodium potassium magnesium-activated adenosine triphosphatase in microsomal fractions of rat and guinea pig brain and on whole homogenates of human brain *Biochem Pharmacol* 1968 *17* 573—000

SELVERSTON A Structure and function of the transverse tubular system in crustacean muscle fibers *Amer Zoologist* 1967 *7* 515

WOODBURY D M Effects of diphenylhydantoin on electrolytes and radiosodium turnover in brain and other tissues of normal hyponatremic and postictal rats *J Pharmacol exp Ther* 1955 *115* 74—80

WOODBURY D M Mechanisms of action of anticonvulsants In *Basic Mechanisms of the Epilepsies* H H Jasper A A Ward Jr and A Pope (Eds) Little Brown and Co Boston 1969 pp 647—681

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Circulatory Control via Vagal Afferents

VI The bleeding bradycardia in the rat, its elicitation and relation to the release of vasopressin

By

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Abstract

SJOSTRAND T *Circulatory control via vagal afferents VI The bleeding bradycardia in the rat its elicitation and relation to the release of vasopressin* Acta physiol scand 1973 *89* 39—50

Bradycardia appears regularly in Sprague Dawley rats on arterial hemorrhage It is usually preceded by an increase of the heart rate of varying duration On an average the bradycardia starts 21 s after the fall in arterial pressure On reinfusion the bradycardia disappears during 10—20 min following a varying course Compression of the inferior caval vein provokes bradycardia which appears and disappears similarly but starts earlier in relation to the pressure fall If blood or saline is intravenously infused simultaneously with the hemorrhage the bradycardia starts later and is less pronounced in relation to the fall in arterial pressure The bradycardia is inhibited by hypophysectomy hydration and infusion of small amounts of ethyl alcohol through the carotid artery A similar bradycardia may be provoked by intra-carotid infusion of vasopressin at maintained arterial pressure

It is concluded that the bleeding bradycardia is elicited by receptors on the low pressure side stimulated by a decrease of the venous return or central blood volume and is a part of a reflex control of the blood volume and distribution The release of vasopressin is stimulated through vagal afferents and by local transmission exerts an excitatory effect on the depressor area in the hypothalamus The significance of the deduced vasopressin releasing reflex under normal and pathological conditions is discu sed

Bradycardia appearing on hemorrhage was found to be completely or partially inhibited by exclusion of vagal afferents via the laryngeus communicans in Sprague Dawley rats (Castenfors and Sjostrand 1972) The allied receptors were supposed to be localized on the low pressure side of the circulation and to be stimulated by a decrease of the central blood volume Discharges increasing on drawing of blood were recorded in the superior laryngeal nerve (Castenfors *et al* 1972) It was also demonstrated that the decrease of the heart rate appears coincidently with an inhibition of the increased sympathetic stimulation on release of the arterial baroreceptor reflex and on elicitation of the cerebral ischemic reflex (Castenfors and Sjostrand 1973) An increase of the antidiuretic activity in plasma was found to

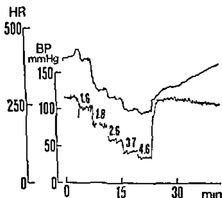


Fig 2

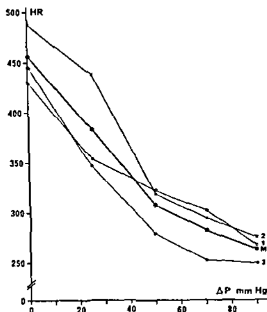


Fig 3

Fig 2 The heart rate on hemorrhage to varying arterial pressure levels The figures indicate the blood volumes which have been drawn off

Fig 3 The relationship between the heart rate and the decrease in arterial pressure on successive hemorrhage in three Sprague Dawley rats and the mean curve

been the same Thus the heart rate may in some rats increase on drawing of up to 12 % of the total blood volume before the bradycardia appears

The relationship between the decrease in heart rate and the arterial pressures

On drawing of blood to varying arterial pressure levels the heart rate is gradually leveled off at different rates (Fig 2) The relationship between the heart rate and the fall in arterial pressure in three rats is shown in Fig 3 The curves fit a polynominal equation obviously at small and large blood losses other factors have an influence

The time relation between the decrease in heart rate and arterial pressure

The latency time for the heart rate decrease on drawing of blood on the arterial side varied between 9 and 48 s in 15 animals (mean 21 s) The great individual differences are dependent upon the appearance of a preceding increase of the heart rate The lower value signifies the latency time without obvious interference with the initial antagonistic effect Usually the initial increase and as a consequence also the latency time become more pronounced during an experiment



Fig 4 Heart rate and arterial pressure on drawing of 26 and 3 0 ml blood from the femoral artery and on decreasing the venous return by partial occlusion of the inferior caval vein (CC) Note that the heart rate decreases practically simultaneously with the decrease of the arterial presurse in the latter case but after a latency time in the former

Elicitation of bradycardia on a decrease of the venous return

Fig 4 shows a comparison between the effect on the heart rate and arterial pressure of a sudden decrease of the venous return by compression of the inferior caval vein and of a rapid blood loss by drawing of blood through a catheter in the femoral artery The records are very similar but after a brief initial rise the heart rate begins to decrease in the former case 3 s and in the latter 18 s after the fall in arterial pressure A shorter latency time in relation to the fall in arterial pressure on venous occlusion was seen also in 3 other rats

The effect of increased venous return simultaneously with drawing of blood from the arterial side

Blood has been drawn off from the femoral artery to a fixed blood pressure alternately with and without infusion of saline or blood through a catheter inserted close to the heart via the jugular vein Fig 5 and 6 demonstrate that the bradycardia ap



Fig 5 Heart rate on continued drawing of blood to an arterial pressure of 40 mm Hg with (large arrows) and without infusion of saline through a catheter inserted in the jugular vein Note that the heart rate drops more slowly and stops at a higher level during the iv infusion On stopping of the infusion the heart rate decreases further

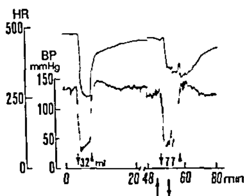


Fig 6 Heart rate on hemorrhage with (large arrows) and without infusion of blood under the same conditions as in Fig 5

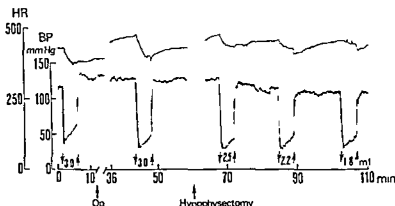


Fig 7 Heart rate on drawing of blood to an arterial pressure of 40 mm Hg before and after hypophysectomy For further description of the experiment see the text

pears later and after a more pronounced initial increase in the heart rate on venous infusion Furthermore the heart rate levels off at a higher frequency On stopping of the infusion the heart rate decreases at a constant or increasing arterial pressure The same results were obtained in four further experiments

The effect of hypophysectomy

Fig 7 demonstrates an experiment with drawing off blood before and after hypophysectomy After exposing the hypophysis by boring a hole in the sphenoidale blood is drawn with the same effect on the heart rate as before the operation The hypophysis is then gently removed without opening the *diaphragma sellae* Blood is drawn again after five minutes The decrease of the heart rate is only half of that before hypophysectomy On subsequent bleedings and reinfusions the heart rate decreases less and less on drop of the arterial pressure and decreases markedly on re infusion of the blood On opening of the diaphragm and removing the stalk the heart

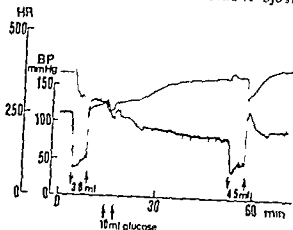


Fig 8 The heart rate response on drawing and reinfusion of blood before and after infusion of 10 ml isotonic glucose solution The response is inverted half an hour after the infusion

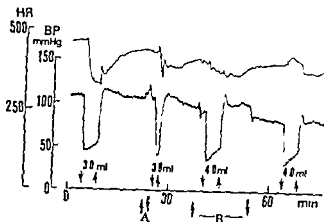


Fig 9 The change of the heart rate on hemorrhage before and after infusion of 10 % ethyl alcohol in isotonic NaCl solution in the peripheral carotid artery at a rate of 0.1 ml/min At A a short infusion repeated at B The heart rate response after infusion at B is opposite to that before Note that the blood volume drawn to reach a fixed arterial pressure is larger after the ethanol infusion

rate response is rapidly changed to the opposite to that before hypophysectomy In seven animals the same results were obtained

The effect of hydration

In order to inhibit the release of vasopressin from the hypophysis an infusion of 10 ml of an isotonic or hypotonic glucose solution was performed in 4 and of an hypotonic NaCl solution in 2 animals The effect of hemorrhage on the heart rate before and after the infusions is demonstrated in Fig 8 The bradycardia has disappeared 35 min after the infusion and the reaction is reversed as after hypophysectomy If the same blood volume is drawn and reinfused repeatedly after infusion of the solution a gradual disappearance of the bradycardia has been observed After 1 h or more the bradycardia may appear again Infusion of the same volumes of isotonic NaCl solution may provoke a slower onset of the bradycardia but does not inhibit it

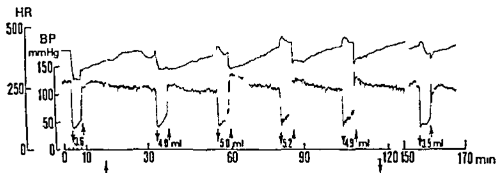


Fig 10 A similar experiment as in Fig 9 on another rat with infusion of 1 % ethyl alcohol in isotonic NaCl solution at a rate of 0 047 ml/min between the large arrows. The reaction is reversed 35 min after stopping the infusion

The effect of infusion of ethyl alcohol in the carotid artery

Ethyl alcohol diluted to 1 and 10 % respectively with isotonic NaCl solution has been infused via the common carotid artery at a rate of 0 26—0 15 ml/min With the higher concentration the bradycardia on hemorrhage disappears within a few minutes and the reaction is reversed *i e* the heart rate increases with the fall in pressure and decreases on reinfusion (Fig 9) On infusion of the lower concentration the bradycardia disappears gradually thus the conditions are the same as after hypophysectomy and hydration (Fig 10) The inhibition of the bradycardia is also reversible if the infusion is stopped It emerges also from Fig 9 and 10 that the blood volume which has to be drawn to lower the arterial pressure to a fixed value increases with the inhibition of the bradycardia

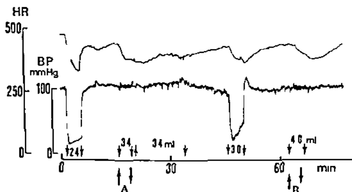


Fig 11 The effect of infusion of 0 1 U vasopressin/ml in isotonic NaCl solution at a rate of 0 15 ml/min via the carotid artery (A) and the jugular vein (B) Blood is continuously drawn from the femoral artery to maintain the arterial pressure during the infusion and then re
The effects are compared with the effect of arterial hemorrhage

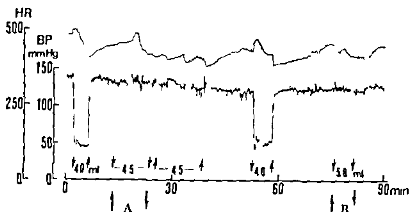


Fig 12 A similar experiment as in Fig 11 on another Sprague Dawley rat demonstrating the initial increase of the heart rate on hemorrhage as well as on intracarotid infusion of vasopressin. At A infusion via the carotid artery at B via the jugular vein

The effect of infusion of vasopressin

Vasopressin in a concentration of 0 1 U/ml in isotonic NaCl solution has been used during 4—5 min at varying rates through catheters inserted through the jugular vein or the common carotid artery in cranial direction In the latter case the carotid sinus has been denervated on the same side Fig 11 and 12 show the effect in two rats and the comparison with the effect of hemorrhage After infusion via the carotid artery the heart rate decreases similarly as on a blood loss In the experiment depicted in Fig 12 the heart rate first increases similarly in both conditions On venous infusion at the same rate the heart frequency decreases but more slowly and to a lesser extent

A decrease of the heart rate on infusion of vasopressin has also been observed after infusion of ethyl alcohol when the bleeding bradycardia has been abolished After hypophysectomy an increase of the heart rate may appear on intracarotid infusion of vasopressin

Intracarotid infusion of vasopressin simultaneously with drawing of blood from the femoral artery to maintain the arterial pressure at the ordinary level has also been performed in prolonged experiments It has been found that about half of the calculated blood volume may be drawn off before the arterial pressure drops

The effect of denervation of the carotid sinus

The bradycardia is provoked also after denervation of the carotid sinus (Fig 13) The figure demonstrates moreover that the initial increase of the heart rate on drawing of blood is not influenced by the denervation

The effect of reinfusion

The increase of the heart rate on reinfusion follows a varying course under different conditions At the beginning of an experiment with repeated blood drawings the

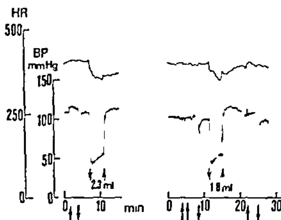


Fig 13 The effect on the heart rate of hemorrhage before and after denervation of the carotid sinus The carotid arteries are occluded between the large arrows After denervation the left and right arteries are occluded in succession The denervation does not significantly influence the response to a blood loss

heart rate first increases rapidly and then slowly Later the initial increase is shortened and succeeded by a decrease After the initial more rapid change the heart rate increases gradually during 10 to 20 min

If blood is not reinfused the heart rate increases more slowly than the arterial pressure

Discussion

The bradycardia on hemorrhage in the rat seems to be effected by a reflex constituting an integral part of the circulatory control Thus it may be elicited on only a small blood loss and displays a quantitative relation to the degree of bleeding It is usually preceded by a rise of the heart rate The bradycardia is elicited also at low arterial pressure The reflex is obviously not elicited by a stimulation of the proprioceptive receptors of the heart as suggested by Öberg and White (1970) with regard to the bradycardia sometimes seen on bleeding in the cat

The experiment with varying of the venous return at maintained arterial pressure is consistent with the assumption that the bradycardia is elicited by a decrease of the venous return or of the central blood volume This presumption is furthermore favoured by the differences between the restitution of the heart rate and the arterial pressure on reinfusion

A direct connection between the bradycardia and the release of vasopressin emerges from the following observations 1) The bradycardia is inhibited by section of vagal afferents as is the release of vasopressin on hemorrhage (Castenfors and Sjostrand 1972) 2) By section of the laryngeal pathway in Sprague Dawley rats the bradycardia is completely or partially inhibited as is likewise the appearance of the antidiuretic effect of the blood on hemorrhage (Freyschuss and Sjostrand 1973) 3) On repeated blood drawings to a maintained arterial pressure of 40—50 mm Hg until the critical point when blood has to be reinfused to maintain the pressure the heart rate depression (after the initial rapid decrease) follows the same course as the anti

diuretic effect of the blood (Freyschuss and Sjostrand 1973) 4 The bradycardia is inhibited by hypophysectomy 5) It is inhibited by hydration 6) It is inhibited by small amounts of ethyl alcohol infused via the carotid arteries, which is known to inhibit the release of vasopressin 7) A bradycardia with similar appearance may be provoked by intracarotid infusion of vasopressin at maintained ordinary arterial pressure The observation that the bradycardia appears during rapid bleeding before the antidiuretic effect of the blood has reached a substantial level indicates that it is provoked in direct connection with the release of vasopressin and thus by a local central effect This supposition is also consistent with the observation that an infusion of vasopressin via the central part of the carotid artery is more effective in provoking bradycardia than an intravenous infusion

An effect of vasopressin on the central nervous system may be exerted 1) through neuronal transmission via neuropiles 2) via the cerebrospinal fluid or 3) via the blood in a portal circulation Transmission of hypothalamic secretion products through neuropiles has been observed in amphibians and mammals (ref in Bern 1966) Transmission also via the cerebrospinal fluid is possible and has been suggested earlier (ref in Ranson and Magoun 1939) Recently Varma *et al* (1969) provoked bradycardia in dogs by injection of 1 U vasopressin in the central ventricles A portal circulation is established between the hypothalamus and the anterior pituitary lobe but not with the posterior A passage of vasopressin from the blood to the liquor in the third ventricle is possible because of the absence of the blood brain barrier in the posterior pituitary lobe the tuber cinereum and the hypophyseal stem (ref in Davson 1967) However in this instance there would be a direct relation between the blood concentration of vasopressin and the bradycardia which is not the case at the initial stage of a blood loss It thus seems most plausible that the central effect of vasopressin is exerted via direct neuronal transmission or via the cerebrospinal fluid The possibility of imitating bleeding bradycardia by intracarotid infusion of vasopressin and the slow disappearance of the bleeding bradycardia on reinfusion are consistent with transmission through the cerebrospinal fluid

The depressive cardiovascular effect concomitantly with the release of vasopressin may be explained by the stimulation of the depressor area in the hypothalamus (Ranson and Magoun 1939 Hess 1948 Folkow *et al* 1959) This is localized to the anterior part as is the supraoptic nucleus which according to prevalent opinion is directly engaged in the production and release of vasopressin Activation of the anterior hypothalamus by a number of drugs may also result in a concomitant release of vasopressin (Walker 1957) It thus seems reasonable to explain the depressive effect on heart rate and arterial pressure by a stimulation of the depressor area through a release of vasopressin to the liquor or by a concomitant neuronal stimulation from the supraoptic nucleus The reversed cardiovascular effect on blocking of the release of vasopressin would accordingly be explained by the activation of the sympathetic cardiovasomotor centres through the arterial baroreceptor reflexes and the cerebral ischemic reflex It is an open question whether the hypothalamic pressor area is engaged in this adaptation

The advantage of depression of the sympathetic control coincidently with the establishment of redistribution of the blood from the capacity vessels to the central part of the circulation has earlier been propounded (Sjostrand 1973) The capacity of vasopressin to compensate a blood loss on combined central and peripheral action is demonstrated by the intracarotid infusion of vasopression simultaneously with drawing of about half of the total blood volume under maintained arterial pressure at the ordinary level and thus without any interference of the arterial baroreceptor mechanisms or the cerebral ischemic reflex

The significance of the vasopressin releasing reflex for the adaptation of the circulation on hemorrhage has been demonstrated by the experiments on repeated bleeding with maintained arterial pressure to the critical point with and without cutting of the laryngeal nerves alone or together with the vagus trunk (Sjostrand 1973) On prolonged hypotension the vasopressin releasing reflex is abolished (Sjostrand 1973 Freyschuss and Sjöstrand 1973) and the impairment of the circulatory adaptation to hemorrhage is the same as on vagal deafferentation The most reasonable explanation of this is that the hypophysis is exhausted of vasopressin

It is tempting to apply the concept of a central effect of vasopressin to explain the difference between the favourable effect of the intact hypophysis on tolerance to bleeding and the disappointing experience of exogenous administration of vasopressin in oligemic shock On an endogenous release of vasopressin the peripheral vascular effect appears coincidently with stimulation of the cardioinhibitory centre and inhibition of the sympathetic vasomotor centres Thus the peripheral effect does not compete with and potentiate the sympathetic vasomotor activity as on exogenous administration Furthermore on exogenous administration of vasopressin the effect would to some extent depress the simultaneous release of vasopressin and thus the central effect

Vasopressin seems to exert its central nervous effect also under ordinary conditions to judge from the effect of exclusion of the vagal afferents via the laryngeal pathway (Castenfors *et al* 1973) After the excitation evoked by section of the nerves the heart rate and arterial pressure increase In consequence the resistance to acute bleeding is increased (Castenfors and Sjostrand 1973) This phase is later followed by a decrease of the arterial pressure which may be explained by the disappearance of vasopressin from the blood cerebrospinal liquor and the smooth muscle receptors Under these conditions the resistance to bleeding is decreased A blocking of the tonic effect of vasopressin seems also to explain the circulatory effect of alcohol

The combined central and peripheral effects of endogenously released vasopressin under *ordinary conditions seem to balance the arterial pressure and the distribution* of the blood between the capacity vessel and the central vessels By these effects the central blood volume is adapted to the total blood volume as are the arterial pressure and cardiac output In the long term also the antidiuretic effect of vasopressin promotes the balance between the volume and distribution of the blood

The bleeding bradycardia is especially pronounced in Sprague Dawley rats How

ever, also in other animals and in man the heart rate does not increase in parallel with the fall of the arterial pressure on bleeding and a bradycardia combined with a decreased sympathetic tone may appear as in fainting the dumping syndrome and the vaso vagal syndrome

References

BERN H A On the production of hormones by neurons and the role of neurosecretion in neuroendocrine mechanisms In *Symposia of the Society for experimental biology* Nr XX Nervous and hormonal mechanisms of integration Ed by G M Hughes Cambridge University Press 1966

CASTENFORS J and T SJOSTRAND Circulatory control via vagal afferents I Adjustment of heart rate to variations of blood volume in the rat *Acta physiol scand* 1972 *84* 347—354

CASTENFORS J and T SJOSTRAND Circulatory control via vagal afferents IV Integration of the central control mechanisms in the circulatory adaptation to variations of blood volume *Acta physiol scand* 1973 *87* 188—198

CASTENFORS J E KNUTSSON and T SJOSTRAND Circulatory control via vagal afferents II Effects of hemorrhage on afferent discharges via the laryngeal communicans in the rat *Acta physiol scand* 1972 *84* 355—365

CASTENFORS J E KNUTSSON and T SJOSTRAND Circulatory control via vagal afferents *III* Control of arterial blood pressure by afferents via the laryngeal communicans with special regard to the restitution after hemorrhage *Acta physiol scand* 1973 *87* 176—187

DAVSON H *Cerebrospinal fluid* J & A Churchill London 1967

FOLKOW B B JOHANSSON and B ÖBERG A hypothalamic structure with a marked inhibitory effect on tonic sympathetic activity *Acta physiol scand* 1959 *47* 267—270

FREYSCHUSS U and T SJOSTRAND Release of vasopressin on repeated hemorrhages and its connection with the circulatory adjustment in the rat In manuscript

W R Die funktionelle Organisation des vegetativen Nervensystems Schwabe & Co Basel 1948

BERG B and S WHITE The role of vagal cardiac nerves and arterial baroreceptors in the circulatory adjustments to haemorrhage in the cat *Acta physiol scand* 1970 *80* 395—403

LANSON S W and H W MAGOUN The Hypothalamus *Ergebn Physiol* 1939 *41* 56—163

JOSTRAND T Circulatory control via vagal afferents V Impairment of the circulatory adjustment to hemorrhage by vagal deafferentation and prolonged hypotension *Acta physiol scand* 1973 *87* 228—239

VARMA S B P JAJU and K P BHARGAVA Mechanism of vasopressin induced bradycardia in dogs *Circulat Res* 1969 *24* 787—792

WALKER J M Release of vasopressin and oxytocin in response to drugs In Heller (Ed) *The Neurohypophysis* Butterworths London 1957 221—229

Acta physiol. scand. 1973. 89. 51—60

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Histaminuria after Parenteral L-histidine Administration in Man

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Abstract

DÖHLEN, H., H. NORNES, O. SJAASTAD and Ö. V. SJAASTAD. *Histaminuria after parenteral L-histidine administration in man.* Acta physiol. scand. 1973. 89. 51—60.

L-histidine was given i.v. to 4 healthy male individuals. The dosage was 2.5, 5.0 and 7.5 g of the free amino acid, and the total number of experiments 9. The urinary excretion of histamine was estimated with 3 different methods: Dunér and Pernow's method (ion-exchange + bioassay), an enzymatic isotopic assay (Snyder *et al.* 1966) and an autoradiographic method. No definite increment in urinary histamine excretion followed the 2.5 g dose, whereas a significant increment followed the 5.0 and 7.5 g dosages. The excess histamine was excreted during the first 4 h after L-histidine loading. The excess urinary histamine probably has its origin in the tissues of the body. The extra endogenous histamine is probably formed as a consequence of the L-histidine loading.

In several species, *e.g.* the rat (Gustafsson *et al.* 1957, Bjurö *et al.* 1963), it has been demonstrated that *in vivo* formation of histamine takes place.

The problem of histamine formation in man is a little more controversial. Both foetal and adult tissues possess a capacity to form histamine in experiments *in vitro* (see Lindell and Westling 1966). Thus, the production system for histamine in man seems to be intact. Nevertheless, great difficulties have been encountered in trying to prove that histamine formation really takes place in man *in vivo*. In experiments with parenteral administration of radioactive L-histidine, one has not been able to demonstrate histamine formation *in vivo*, since no trace of radioactive histamine or radioactive histamine metabolites has been found in the urine (Dennis *et al.* 1961, Westling, personal communication).

However, in some non-isotopic studies in which a much larger dosage of L-histidine had been administered *orally*, an increment in urinary excretion of histamine was found (Irvine *et al.* 1959, Oates *et al.* 1962, Sjaastad 1966 a). Experiments with oral L-histidine after attempted sterilization of the gut have given equivoca

TABLE I Urinary excretion of histamine on control day (A) and on day of intravenous infusion of L-histidine (B) (uncorrected for losses during extraction procedure)

Histamine μg base per hour

Subj no	Pre test* period	0–4 h	4–8 h	8–12 h	12–24 h	L-histidine dose
1 A	0.6	0.7	0.7	1.0	0.8	
B	0.3	9.8	0.5	0.7	1.1	7.5 g
2 A	0.4	0.3	0.4	0.9	0.3	
B	0.5	7.0	0.8	0.3	0.9	7.5 g
2 A	0.8	0.7	0.5	0.8	0.7	
B	0.7	3.7	0.8	0.7	0.7	7.5 g
1 A	1.1	2.1	1.9	0.8	0.3	
B	0.5	3.1	1.7	1.0	2.1	5 g
2 A	0.3	0.3	0.3	0.2	0.3	
B	0.7	2.1	0.3	0.7	0.5	5 g
3 A	0.7	0.2	0.2	0.7	0.7	
B	0.2	3.8	0.2	1.9	0.3	5 g
4 A	0.2	0.6	0.2	0.4	0.2	
B	0.5	2.0	0.7	0.1	0.1	5 g
2 A	0.5	0.5	0.5	0.5	0.4	
B	0.5	0.6	0.6	0.5	0.5	2.5 g
4 A	0.2	0.3	1.0	0.5	0.1	
B	0.4	0.8	0.6	0.5	0.3	2.5 g

* Pre test period: last two hours before start of L-histidine infusion

as to whether the extra histamine in urine after loading with L-histidine derives from histamine formed in the tissues or intraluminally in the gut (Irvine *et al.* 1959, Oates *et al.* 1962).

If the hypothesis were correct that this histamine is formed in the tissues, an increment in the urinary excretion of histamine should also follow parenteral L-histidine administration. The principal aim of the present study was to investigate whether such increase in urinary histamine-like activity follows parenteral L-histidine administration and next to examine whether the excess histamine-like activity is due to histamine.

Material and methods

Four healthy male volunteers, aged 20—41 years, were studied. The dose of L-histidine monohydrochloride corresponds to 2.5 g of the free amino acid in the first 2 expts., to 5 g in the next 4 expts. and to 7.5 g in the last 3 expts. (Table I).

A 5 % solution of L-histidine monohydrochloride[1] (adjusted to pH 7.0 by addition of NaOH) was infused i.v. in the course of 45 min, the infusion starting at 9 a.m. There were no untoward effects of these infusions whatsoever, in particular they were not accompanied or followed by headache. Urine collection was carried out from 7 a.m. to 9 a.m. for estimation of pretest histamine excretion. With the start of infusion as zero point, separate urine collection was then carried out from 0—4 h, 4—8 h, 8—12 h and from 12—24 h. A control study without L-histidine loading was carried out on each occasion at least 1 week apart from the

[1] The L-histidine solution was kindly supplied by AB Astra, Södertälje, Sweden.

L histidine study the urine collection starting at exactly the same time of the day

On test as well as control days the test subjects were fasting the fast starting 12 h prior to the start of urine collection and lasting throughout the period of urine collection Adequate diuresis was maintained by small amounts of water by mouth Sufficient 1 N hydrochloride acid was added to the urine flasks to keep the pH at approximately 2 0 The bottles were kept at +4 C during urine collection

The method of Duner and Pernow (1956) was employed for the estimation of histamine and conjugated histamine in urine Some minor modifications were made (Ö V Sjaastad 1967) thus obviating most of the difficulties encountered with the original method (Wetterquist and White 1970) For the reproducibility of the method it is of the utmost importance that the eluates are close to isotonic because of the final biological assay With the original method strongly hypertonic eluates were often obtained Osmometric analysis (Knauer) did however show that eluates were close to isotonic with the modifications introduced (Ö V Sjaastad 1967)

The figures for histamine in the present study refer to histamine base and represent the mean of duplicate analyses

In healthy adults the following unweighted values for urinary histamine were found (uncorrected for losses during recovery) 12 6 μg base/24 h±6 3 (mean±S D) and range 2—31 μg and conjugated histamine 30 0±23 6 (1—99) Details regarding the method are published elsewhere (Sjaastad 1967) The mean recovery of small amounts of added histamine diphosphate was 65 in the present series

Other methods used for the identification of the bioactive substance

Aliquots of the 0—4 h urine portions which contained most histamine like activity after L histidine loading (7 5 g) were run through Amberlite IRC 50 columns and the histamine content of the eluates was estimated with other methods

1) The specific enzymatic isotope assay of Snyder *et al* (1966)

2) An autoradiography bioassay method

Great difficulties were encountered in trying to establish by regular paper chromatographic technique the identity of the minute quantities of histamine like activity in the eluates with histamine Since bioassay is far more sensitive than staining methods when histamine is concerned we have used an autoradiographic procedure the main features of which are the following A small quantity of the eluate containing 0 25—0 75 μg histamine base as determined by bioassay was spotted on a chromatogram (Whatman No 1)—3 chromatograms from each eluate—together with such a minute quantity of ^{14}C histamine dihydrochloride (i e 5—10 ng) as not to interfere with the final assay of histamine ^{14}C-histamine dihydrochloride labelled in the 2 position of the ring with specific activity 54 mCi/mmol (purchased from The Radiochemical Centre Amersham England) was used After bidimentional runs (descending technique) Kodak x ray films were exposed to the chromatograms for 6—8 weeks and the area of the unstained paper chromatogram corresponding to the dark area on the film was cut out The histamine contained in it was eluted with small amounts of 0 9% NaCl (final volume 1 5—2 5 ml) and was then assayed on the isolated guinea pig ileum

The following solvents were used System 1 n butanol acetic acid-distilled water (4 1 1) System 2) n butanol saturated with 20% NH_4OH

The bioactive sidechain methylhistamines (Vartiainen 1935) are located rather far away from the histamine spot with these solvent systems (Appel and Werle 1959) and do not represent any source of contamination in this procedure

With the chromatographic technique used histamine is well separated from the other histamine metabolites with the exception of 1 4 methylhistamine which runs just in front of histamine when system 2 is used (Eliassen 1969)

The biological activity of 1 4 methylhistamine on guinea pig ileum is about 1/170 of that of histamine (Lee and Jones 1949) and the methylhistamine/histamine ratio in urine will therefore be one factor determining whether methylhistamine represents a source of error

The excretion of 1 4 methylhistamine in experiments with intravenous L-histidine loading is not known An approximate estimate can however be obtaind from the available information On a mixed diet the spontaneous methylhistamine/histamine ratio was found to be between 3 7 and 9 0 (Granerus 1968 Granerus *et al* 1968) On oral L-histidine loading a methylhistamine/histamine ratio in urine of 3 8 was found

This roughly estimated 1 4 methylhistamine in urine may produce a biological activity on guineapig ileum amounting to between 2 2 and 5 4% of that of histamine (corresponding to methylhistamine/histamine ratios 3 7 and 9 0) under ordinary circumstances and oral L-histidine loading The interrelationship of 1 4 methylhistamine and histamine may be somewhat different in our experiments but probably not markedly so

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Histamine µg base per hour

Subj no	Pre test* period	0—4 h	4—8 h	8—12 h	12—24 h	L-histidine dose
1 A	0 6	0 7	0 7	1 0	0 8	
B	0 3	9 8	0 5	0 7	1 1	7 5 g
2 A	0 4	0 3	0 4	0 9	0 3	
B	0 5	7 0	0 8	0 3	0 9	7 5 g
2 A	0 8	0 7	0 5	0 8	0 7	
B	0 7	3 7	0 8	0 7	0 7	7 5 g
1 A	1 1	2 1	1 9	0 8	0 3	
B	0 5	3 1	1 7	1 0	2 1	5 g
2 A	0 3	0 9	0 3	0 2	0 3	
B	0 7	2 1	0 3	0 7	0 5	5 g
3 A	0 7	0 2	0 2	0 7	0 7	
B	0 7	3 8	0 2	1 9	0 3	5 g
4 A	0 2	0 6	0 2	0 4	0 2	
B	0 5	2 0	0 7	0 1	0 1	5 g
2 A	0 5	0 5	0 5	0 5	0 4	
B	0 5	0 6	0 6	0 5	0 5	2 5 g
4 A	0 2	0 3	1 0	0 5	0 1	
B	0 4	0 8	0 6	0 5	0 3	2 5 g

* Pre test period last two hours before start of L-histidine infusion

as to whether the extra histamine in urine after loading with L-histidine derives from histamine formed in the tissues or intraluminally in the gut (Irvine *et al* 1959 Oates *et al* 1962)

If the hypothesis were correct that this histamine is formed in the tissues an increment in the urinary excretion of histamine should also follow parenteral L-histidine administration The principal aim of the present study was to investigate whether such increase in urinary histamine like activity follows parenteral L-histidine administration and next to examine whether the excess histamine like activity is due to histamine

Material and methods

Four healthy male volunteers aged 20—41 years were studied The dose of L-histidine monohydrochloride corresponds to 2 5 g of the free amino acid in the first 2 expts. to 5 g in the next 4 expts., and to 7 5 g in the last 3expts. (Table I)

A 5 % solution of L-histidine monohydrochloride[1] (adjusted to pH 7 0 by addition of NaOH) was infused i v in the course of 45 min, the infusion starting at 9 a.m. There were no untoward effects of these infusions whatsoever in particular they were not accompanied or followed by headache Urine collection was carried out from 7 a.m to 9 a.m. for estimation of pretest histamine excretion. With the start of infusion as zero point, separate urine collection was then carried out from 0—4 h 4—8 h 8—12 h and from 12—24 h A control study without L-histidine loading was carried out on each occasion, at least 1 week apart from the

[1] The L-histidine solution was kindly supplied by AB Astra Sodertalje Sweden

TABLE II Determination of histamine with 3 different methods Two 0–4 h urine samples after 7 5 g L-histidine orally (histamine values in μg base/4 h)

Subject no	Duner and Pernow s method*	Autoradiography + bioassay **	Specific enzymatic procedure
1	39	26	15
2	28	20	20

* Uncorrected for losses during extraction procedure
** See Material and methods for details
*** *Snyder et al 1966*

methylhistamines since these are not inactivated by diamine oxidase (Kapeller Adler and Iggo 1957)

2 The results of the histamine determination with the enzymatic isotopic method of Snyder *et al* (1966) are shown in Table II The values obtained with this method *tend to be lower than those obtained with Duner and Pernow s method*

Also with the enzymatic method the histamine excretion per hour during the first 4 h after L-histidine loading was far higher than the excretion in any of the control periods *i e* 5 0 and 3 75 μg base respectively in the 2 expts

3 The results of the autoradiographic studies are shown in Table II It is evident that although the figures obtained with this method are lower than those obtained *with* Duner and Pernow s method the histamine excretion following intravenous L-histidine loading is far above that in any control period The values obtained with this method do not seem to be lower than those obtained with the specific enzymatic method This seems somewhat peculiar when considering the losses during paper chromatography and elution with the autoradiographic method The values obtained with the autoradiographic method are probably underestimated (*cf* methods) The values obtained by Snyder *et al s* method may therefore also be too low

Two different methods have thus shown that the extra histamine in urine is identical with histamine

Could the excess urinary histamine derive from sources other than the tissues?

The following sources for the excess urinary histamine in our experiments should be considered the L-histidine infusion solution the urine itself (infection) and the lumen of the gastrointestinal tract

1) The L-histidine infusion solution L-histidine is known to contain varying quantities of histamine (*e g* Waton 1963) Each batch of the L-histidine infusion solution was therefore analyzed for its histamine content by bioassay The highest quantity of histamine found was 6 0 μg base in a 7 5 g L-histidine solution Of intravenously injected histamine approximately 3 % is excreted unchanged in the urine (Lindell and Westling 1966 Granerus *et al* 1968) The contribution to the histamine from the L-histidine dosage containing most histamine will not amount to more than 0 2 μg *i.e.* a quantity insufficient by far o

crement in urinary histamine in our experiments

2) Urinary infection may cause histaminuria (Bjuro *et al* 1961) To exclude this possibility urine from subjects no 1, 2 and 4 was cultured on blood and lactose agar for 18 h No bacterial growth whatsoever was detected

Urine for culture was taken from urine samples which had been retained in the bladder for exactly the same length of time as the urine samples which contained the excess histamine *i e* for 4 h

3) Histamine originating from the lumen of the gastrointestinal tract

Could the excess histamine derive from histamine formation intraluminally in the bowels? In their review of histamine metabolism in man Lindell and Westling (1966) stated that in order to prove that endogenous histamine formation really occurred in man after parenteral L histidine administration amongst other things the following condition must be met the gut had to be sterilized intraluminally Otherwise the following chain of events might take place The parenterally administered L histidine might be excreted into the gastrointestinal lumen to a certain extent and decarboxylated within the lumen the histamine being absorbed and excreted partly as such in the urine

Indeed Irvine *et al* (1959) found a marked difference in histaminuria following oral L histidine loading before and after reduction of the bacterial content of the gut by means of succinylsulphathiazole We did not attempt to bringt about any sterilization of the gut in the present study the main reason for this being that vari s experiments indicated that no pronounced formation of histamine takes place rmally in the lumen of the human gut

The faecal histamine levels in man are extremely low (Sjaastad 1967) *In vitro* incubation experiments with L histidine and faeces showed only a moderate formation of histamine (Sjaastad 1967)

Obviously the observed formation of histamine could be erroneously low if the catabolism of formed histamine was high The catabolism *in vitro* of histamine by human faeces did not appear to be particularly high either (Sjaastad and Sjaastad 1971)

Another strong indication that the intraluminal histamine production *in vivo* normally is only moderate is the finding of uniformly low levels of conjugated histamine in urine in healthy individuals (Sjaastad 1967) If histamine were formed intraluminally to a large extent one would expect that the urinary conjugated histamine would rise Whenever histamine is given orally (Roberts and Adam 1950 Sjaastad 1966 b) or formed in the gastrointestinal lumen as in myotonic dystrophy (Sjaastad 1967) and chronic pancreatitis (Sjaastad 1969) conjugated histamine is excreted in large amounts and the conjugated histamine/histamine ratio increases markedly (Table III)

The urine samples from test subjects 1 and 2 who exhibited the most pronounced response with regard to urinary histamine showed no corresponding increase in conjugated histamine *i e* there was a low conjugated histamine/histamine ratio (Table III) It is evident that the pattern found when L histidine is given orally or

TABLE III Conjugated histamine/histamine ratio in urine in various conditions

	No of patients/ test subjects	Mean excretion µg base/24 h		
		Histamine *	Conj hist *	Ratio conj hist./hist
Increased intestinal formation of histamine				
Chronic pancreatis	10	24	577	24
Myotonic dystrophy	20	19	318	17
Histamine administered to the gastrointestinal tract				
Histamine diph orally 200 mg	8	22	1090	49
Histamine diph intestinally 200 mg	8	11	2270	208
Histamine diph rectally 200 mg	3	12	990	83
L-histidine monohydrochloride administration				
Orally 10 g	8	44	53	1 2**
Intravenously 10 g	2	55	33	0 6
Controls	37	13	30	2 4
	17***	12	22	1 9

* Unweight values
** One individual had a pre test increased excretion of conjugated histamine (240 µg/24 h versus 250 µg/24 h on the test day) If this patient is excluded the mean ratio would be 0 57
Data compiled from Sjaastad 1966a b 1967 & 1969 except for
*** Dunér and Pernow 1956

intravenously is quite different from the afore mentioned patterns Whereas the urinary conjugated fraction seems to be uninfluenced histamine itself rises (Table III)

Since the conjugated histamine/histamine pattern is identical when L histidine is administered intravenously and orally this points to a common origin of the excess histamine excreted in the urine in these 2 experimental conditions Since histamine in the gut gives rise to increased urinary excretion of conjugated histamine associated with no significant rise in histamine—a pattern entirely different from that accompanying L histidine loading—the histamine found in the urine in our experiments probably does not originate in the lumen of the gut it probably originates in the tissues

Due to the experimental conditions dietary L histidine and histamine can be excluded as the source of the extra urinary histamine (see Materials and Methods)

Discussion

In some previous studies in man it has been found that administration of L histidine is followed by an increment in urinary histamine excretion (Irvine *et al* 1959 Oates *et al* 1962 Sjaastad 1966 a) whereas in other studies no such increase was found (Duner *et al* 1961 Granerus 1968)

In the studies with a positive result in this respect either a high dosage of histamine *i e* approximately 10 g L *histidine monohydrochloride* (Oates *et al* 1962 Sjaastad 1966 a) was given or hourly urine collection was combined with a lower

dosage 4 5 g (Irvine *et al* 1959) Common to the studies with negative results was a relatively low dosage, *i e* 5 g combined with a 24 h urine collection Under these last mentioned experimental conditions the small increment in histamine excretion may easily be swamped due to the considerable day to-day intraindividual variation in histamine excretion that takes place on an ordinary mixed diet (Lindell and Westling 1966 Sjaastad 1967) This is also evident in the present study, where 5 g L histidine dosages led to a histamine excretion exceeding the normal range in only 1 of 4 expts when considering the 24 h excretion, whereas an increment in excretion was quite obvious in all experiments when comparing the 0—4 h excretions

The fact that 5 different methods (Roberts and Adam s method Oate *et al* s fluorometric method Duner and Pernow s method and in the present study an autoradiographic method and a specific enzymatic isotopic assay (Snyder *et al* 1966)) all show an increased histamine excretion indicates that the substance tested is identical with histamine both with oral and parenteral L-histidine loading

As previously shown it appears both by exclusion and by analogy, that the extra histamine most likely has its origin in the tissues of the body This may be brought about by 3 different mechanisms 1 formation of histamine 2 liberation of previously formed histamine 3 reduction of histamine catabolism

Oates *et al* (1962) showed that the effect of oral L histidine on urinary histamine excretion is probably a specific one since the hydrochloride salt of another basic amino acid *i e* lysine did not provoke a similar response Of course it cannot be totally excluded from this finding that L histidine causes histamine liberation or a change in catabolism but this appears most unlikely It seems reasonable to assume that histamine formation is the underlying mechanism of the histaminuria both in oral and intravenous loading experiments

It is of interest to compare the increment in urinary histamine excretion with different L histidine dosages and modes of administration Data from such experiments are compiled in Table IV Since there may be a non linear relationship between the L histidine dosage and the histamine response the L histidine dosages may be

TABLE IV Increment in urinary histamine loading on oral and i v L histidine loading

Mode of L-histidine administration	Mean dose of L histidine (g)	Mean increment in urinary histamine (μg)	Method	References
Oral	4 5	12 1	I	Irvine *et al* 1959
Oral	7 2	31 8	II	Oates *et al* 1962
Oral	7 4	39 4	III	Sjaastad 1966 a
Intravenous	5 0	11 8	III	Present report
Intravenous	7 5	40 2	III	Present report

Methods I Roberts and Adam 1950
II Oates *et al* 1962
III Duner and Pernow 1956

The figures have been corrected for recovery according to the recoveries given for the 3 different methods.

critical Fortunately in Irvine *et al* s study (1959) and in Oates *et al* s study (1962) the dosages were rather similar to those in our studies

The histamine response to 5 g (4 5 g) L histidine by i v and oral route seems to be of the same order of magnitude The same applies to the 7 5 g (7 2—7 4 g) L-histidine dosage by i v and oral routes

The percentage of L histidine that has been converted to histamine may be calculated roughly Approximately 3 % of parenterally administered histamine is excreted unchanged in the urine (Lindell and Westling 1966) On the assumption that formed and exogenous histamine are catabolized to approximately the same extent and that histamine formation is really taking place in these experiments the following figures will be obtained With a dosage of approximately 7 5 g L histidine roughly a little less than 0 02 % was transformed to histamine The corresponding figure with the 4 5—5 0 g L histidine dosage was approximately 0 008 %

Although only a small percentage of administered histamine is excreted unchanged urinary histamine appears to reflect the histamine turnover in man rather well (Lindell and Westling 1966) Nevertheless estimation of the major histamine metabolites *i e* 1 4 methylimidazoleacetic (MeImAA) acid and 1 4 methylhistamine (and imidazoleacetic acid) might be more meaningful in this respect than estimation of histamine only Granerus (1968) found no definite increment in urinary MeImAA excretion after oral L-histidine loading to healthy test subjects This may seem to disprove that histamine formation follows L-histidine administration in man There may however be another explanation for this finding

Less than 0 01 % of a 3 7 g L-histidine dosage as used by Granerus will probably be converted to histamine and of the histamine formed probably about 50—60 % *i e* approximately 180 μg will be excreted as MeImAA in the urine Such a small increment in MeImAA would easily be masked in a 24 h urine sample since the control excretion (Granerus 1968) ranges between 3500 and 5900 μg/24 h

It may seem somewhat strange that the excess histamine excretion in such loading experiments culminates so quickly and seems to be over in 4—5 h (Irvine *et al* 1959 and present experiments) Experiments with parenterally administered radioactive histamine have shown that most of the unchanged histamine is excreted into the urine in the course of 6 h (*e g* Lindell and Westling 1966) Nevertheless the rapid formation and excretion of histamine on L-histidine loading may point to the kidney as the organ of production It seems more unlikely that the gastrointestinal wall is the organ of production since the formation of histamine is of the same magnitude whether L-histidine is administered orally or intravenously

In conclusion it has been demonstrated that intravenous L histidine loading seems to be followed by histaminuria probably secondary to histamine formation Whether this histamine is physiologically important or just a wasteproduct formed in the kidneys is another question

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Referens

APPEL W and E WERLE Nachweis von Histamin N Dimethyl histamin N acetylhistamin und Acetylcholin in spinacia oleracea *Arzneimittel Forsch* 1959 *9* 22—26
BJURO T S LINDBERG and H WESTLING Observations on histamine in pregnancy and the puerperium *Acta obstet gynec scand* 1961 *40* 152—173
BJURO T H WESTLING and H WETTERQUIST On the evaluation of histamine formation and catabolism in rats by analysis of the urine *Arch int Pharmacodyn* 1963 *144* 337—346
DEMIS D J M D WALTON D WOOLLEY N WILNER and G McNEIL Further studies of histidine and histamine metabolism in urticaria pigmentosa *J invest Derm* 1961 *37* 513—521
DUNER, H and B PERNOW Urinary excretion of histamin in healthy subjects *Scand J clin Lab Invest* 1956 *8* 296—303
DUNER H S O LILJEDAHL and B PERNOW Does the urinary excretion of imidazoleacetic acid reflect the endogenous histamine metabolism in man? *Acta physiol scand* 1961 *51* 41—46
ELIASSEN K Metabolism of [14]C-histamine in domestic animals I Goat *Acta physiol scand* 1969 *76* 172—181
GRANERUS G Effects of oral histamine histidine and diet on urinary excretion of histamine methyl histamine and 1 methyl 4 imidazoleacetic acid in man *Scand J clin Lab Invest* 1968 *22* Suppl 104 49—58
GRANERUS G H WETTERQUIST and T WHITE Histamine metabolism in healthy subjects before and during treatment with aminoguanidine *Scand J clin Lab Invest* 1968 *22* Suppl 104 39—48
GUSTAFSSON B G KAHLSON and E ROSENGREN Biogenesis of histamine studied by its distribution and urinary excretion in germ free and not germ free rats fed a histamine free diet *Acta physiol scand* 1957 *41* 217—228
HORLINGTON M J C KOLTHAMMER and R LAZARE Correlation of urinary histamine excretion and 24 h urine volumes in rats and man *J Pharmacol Pharm* 1970 *22* 793—795
IRVINE W T H L DUTHIE and N G WATON Urinary output of free histamine after a meat meal *Lancet* 1959 *I* 1061—1064
KAPELLER ADLER R and B IGGO Histamine and its derivatives in human urine *Biochim biophys Acta* (Amst) 1957 *25* 394—402
[illegible] H M and R G JONES The histamine activity of some β aminoethyl heterocyclic nitrogen compounds *J Pharmacol exp Ther* 1949 *95* 71—78
[illegible]NDELL S E and H WESTLING Histamine metabolism in man In *Handbook of experimental pharmacology* Edited by O Eichler and A Farah Springer 1966 *18* 734—788
OATES J A E MARSH and A SJOERDSMA Studie on histamine in human urine using a fluorometric method of assay *Clin chim Acta* 1962 *7* 488—497
ROBERTS M and H M ADAM New method for the quantitative estimation of free and conjugated histamine in body fluids *Brit J Pharmacol* 1950 *5* 526—541
SCHAYER R W Catabolism of histamine *in vivo* In Eichler O and Farah A *Handbuch der experimentellen Pharmakologie* 1966 *18* pp 672—683 Springer Berlin New York
SJAASTAD O Histamine formation after oral L histidine in health and dystrophia myotonica. *Acta med scand* 1966 a *180* 581—588
SJAASTAD O Fate of histamine and N acetylhistamine administered into the human gut *Acta pharmacol* (Kbh) 1966 b *24* 189—20[illegible]
SJAASTAD O *Studies on the metabolism of histamine in dystrophia myotonica* Thesis 1967 Universitetsforlaget Oslo
SJAASTAD O Increased enteric formation of histamine in chronic pancreatitis *Acta med scand* 1969 *186* 125—133
SJAASTAD Ø V Determination and occurrence of histamine in rumen liquor of sheep *Acta vet scand* 1967 *8* 50—70
SJAASTAD Ø V and O SJAASTAD Rupture of the imidazole ring of histamine and N acetyl histamine by human intestinal contents *Acta pharmacol* (Kbh) 1971 *30* 366—371
SNYDER, S H R J BALDESSARINI and J AXELROD A sensitive and specific enzymatic isotopic assay for tissue histamine *J Pharmacol exp Ther* 1966 *153* 544—549
VARTIAINEN A The action of certain new histamine derivatives *J Pharmacol exp Ther* 1935 *54* 265—282
WATON N G Histamine as an impurity in samples of histidine *J Pharm Pharmacol* 1963 *15* 514—518
WESTLING H Personal communication to the authors
WETTERQUIST H and T WHITE Bioassay of histamine in human urine An improved method for purification of samples *Scand J clin Lab Invest* 1970 *25* 325—328

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Acid Production in the Isolated Perfused Rat Liver

By

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Abstract

MORLAND J T CHRISTOFFERSEN and J B OSNES *Acid production in the isolated perfused rat liver* Acta physiol scand 1973 *89* 61—67

The release and production of organic acids were studied in isolated perfused rat livers The livers were perfused at different pH with and without ethanol. Liver homogenate pH was measured in certain experiments The results showed that the basal production of acid by the liver was greatest in the early phase of perfusion probably due to the low initial concentration of acid metabolites in the perfusate The acid production declined when perfusate pH was reduced and increased when perfusate pH was raised Ethanol treatment (perfusate concentration 30—45 mM) increased the liver acid production Liver homogenate pH was more reduced after ethanol than after acid addition to the perfusate at the same perfusate pH It is postulated that ethanol metabolism may influence pH sensitive metabolic reactions by the accompanying acid production

Various acid metabolics (lactic acid pyruvic acid amino acids and keto acids) can be released from and taken up by the liver Changes in the liver metabolism may therefore influence the concentration of these acids in blood and thus alter the metabolic component of the acid base equilibrium Our experiments were performed to study the magnitude of the organic acid release and uptake at different conditions The all-over release and production of acid was recorded in isolated rat livers perfused at constant pCO in this preliminary study

Materials and Methods

Animals Male albino Wistars rats (280—300 g b wt) were fasted for 20 h and used as donors of both blood and liver

Liver perfusion Isolated livers (6.9—8 2 g) were perfused at 37 C in an apparatus described previously (Seglen and Jervell 1969) through vena pora with constant perfusion pressure The recirculaing perfusate volume (30 ml) consisted of rat blood diluted with two parts of a modified Schimassek buffer (Seglen and Jervell 1969) containing albumin (bovine fraction V Sigma) The perfusate was oxygenated with moist 95% O_2 and 5% CO_2 Perfusate pH was adjusted by means of 0 5 N $NaHCO_3$ and 0 1 N HCl To avoid anoxia during the operative preparation of the livers the perfusion started in *in situ* with perfusates which were

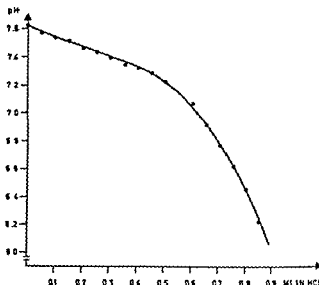


Fig 1 Titration curve of perfusate. Perfusate was recirculated in the perfusion unit in equilibrium with the gas mixture with no liver present. Portions of 0.05 ml 1 N HCl were added and perfusate pH was registrered. The results of one typical experiment are shown.

not recirculated (approximately 4 ml) (Mørland 1971). The pH of these perfusates were the same as the initial perfusate pH in the following perfusion of isolated livers. When ethanol was given it was added after 5 min and infused continuously during the perfusion (1.2 ml/h of 13 % (v/v ethanol). Perfusate ethanol concentration was 30—45 mM (0.15—0.20 %).

None of the livers used showed abnormalities in gross appearance during the perfusion. The mean perfusate flow rate was 1.5 ml/min g liver. In some experiments small liver specimens (100 mg) were taken and frozen immediately in liquid nitrogen.

pH determinations. pH was measured in the perfusate entering the liver with a microelectrode unit (Type E 502 a, pH meter 27 or BMS 3 Digital Acid Base Analyser Type PHM 2b, Radiometer, Copenhagen). In some experiments pH was recorded in perfusate leaving the liver too. To measure the degree of intracellular acidity, samples of frozen liver (100 mg) were rapidly homogenized in ten volumes of an icecold solution containing 145 mM KCl and 10 mM NaCl and pH determined by the microelectrode unit as described elsewhere (Hermansen and Osnes 1972). pH was recorded in these homogenates every 20 s. During the first minutes pH usually declined 0.02—0.03 pH units, followed by slowly increasing pH values.* The lowest pH recorded in each homogenate is given in our results. The liver homogenate pH in several samples taken from the same liver was almost constant during perfusion for 2 h at constant perfusate pH (maximal S.E. ±0.01 pH units). After alteration in pH of the perfusate the pH recordings in homogenate and perfusate were allowed to stabilize (within 3—5 min) before further alterations were made.

Metabolite determination. The Boehringer kits were used to determine perfusate lactate and pyruvate concentration.

Calculations

Acid release from the liver. pCO_2 of the perfusate was constant (approximately 35 mm Hg) in our experiments since perfusate was in equilibrium with 5 % CO_2. Changes in perfusate pH was therefore due to the accumulation of non volatile acids. The amount of acid accumulating in perfusate was calculated from the titration curve obtained with recirculating perfusate (without liver) (Fig. 1).

Acid production of the liver. In another series of experiments perfusate pH was kept at the initial pH value by means of 0.5 N $NaHCO_3$. Since pCO_2 and pH was constant in perfusate as well as in liver homogenates in these experiments, the amount of added bicarbonate was a measure of the total acid production.

* The pH of each sample was stable for at least 1 min in contrast to pH of muscle homogenate samples.

TABLE I Liberation of acid from isolated perfused rat livers Perfusate pH was adjusted to the given value before the liver perfusion started and no additions were given to the perfusates during perfusion except ethanol in some experiments (30–45 mM) 4–8 liver specimens (each 100 mg) were taken from each liver to analysis of liver homogenate pH during the perfusion The amount of moles acid liberated was calculated from the reduction of perfusate pH 3–6 livers were tested at each pH value The mean of values are presented Maximal S E was ±0 05 pH units

Perfusate pH				Amount acid liberated (μmoles/2 h per g liver)
0 h	30 min	1 h	2 h	
7 40	7 27	7 22	7 14	36 4
7.20	7 12	7 10	7 03	13 6
6 85	6 75	6 69	6 66	4 6
6 50	6 50	6 49	6 51	0 0
6 20	6 30	6 35	6 30	−3 6
7 40+Ethanol	6 99	6 82	6 14	81 5

Results

Acid release by the perfused liver The isolated liver from fasted rats perfused at basal conditions liberated acid There was a gradual decrease of perfusate pH from 7 4 to about 7 1 most marked during the first 30 min of perfusion (Table I first line) This release of acid was probably not related to liver hypoxia and anaerobic metabolism since perfusate lactate levels decreased from 1 1 mM to 0 5 mM during the first h of perfusion Furthermore perfusate lactate/pyruvate ratio the mean value of which was 12 did not increase during perfusion No pH decrease was recorded in perfusates recirculating in the apparatus without liver

pH dependency of acid production in the liver Table I shows that the amount of acid liberated was dependent on the initial perfusate pH When perfusate pH was reduced by addition of acid before the perfusion started we recorded a reduction of acid liberation in the subsequent liver perfusion Initial pH values lower than 6 5 was followed by an apparent uptake of acid by the liver (Table I) which may

TABLE II Acid production in isolated perfused rat livers Perfusate pH was adjusted before the liver perfusion and kept at the initial value during the perfusion by addition of 0 5 N NaHCO The amount of bicarbonate added to keep constant pH is given Livers (number in parenthesis) were tested at each pH value no specimens were taken from these livers The mean values±S E are given

Perfusate pH	Total acid production (μmoles NaHCO added per 2 hour per g liver)	Acid production during the second hour of perfusion (μmol s NaHCO added per g liver between 1 and 2 hours of perfusion)
7 60±0 02	140±24 (6)	23 8±2 4 (3)
7 40±0 02	109±13 (6)	19 4±1 8 (3)
7 20±0 04	78±17 (6)	15 5±2 0 (3)
7 40±0 03+Ethanol	215±28 (6)	80 2±6 3 (3)

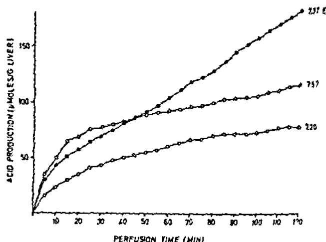


Fig 2 Acid production by isolated perfused rat livers Each point represent the cumulative acid production at the given time Three experiments are presented the perfusate pH (which was constant) is indicated at the curves One perfusion was carried out with the addition of ethanol (●) the other without (○)

represent the buffering capacity of intracellular systems in the liver This uptake of acid could also be demonstrated in experiments in which pH was measured simultaneously in perfusate entering and leaving the liver At perfusate pH about 6 2 due to acid addition pH in perfusate leaving the liver was 6 29 while pH in perfusate entering the liver was 6 20 Other experiments were performed at different constant pH values (Table II) Also in these experiments the acid production was lowered when the pH of the perfusate was set at a lower level The acid production apparently occurred at two different velocities The initial rapid production was followed by a much slower acid formation which was almost constant for at least 2 h of perfusion (Fig 2)

Acid production after ethanol Ethanol is known to produce acid metabolites (Himwich *et al* 1933 Seligson *et al* 1953) We found that ethanol addition increased acid release and total acid production (Table I and II lower lines) and (Fig 2) The slow constant acid production was about four times higher after ethanol than in controls The results presented in Table I indicated that the liver could produce acid even at low perfusate pH when ethanol was given This was verified in separate experiments performed at perfusate pH 6 2 due to ethanol administration In these experiments we found that pH was lower in perfusate leaving the liver (pH 6 18) than in perfusate entering the liver (pH 6 23)

Relationship between perfusate pH and intracellular acidity We compared the relationship between liver homogenate pH (intracellular acidity) and perfusate pH when a) ethanol was oxidized and b) when the changes in perfusate pH were due to addition of HCl to the perfusate Fig 3 shows that homogenate pH varied to a smaller extent than perfusate pH The changes in intracellular acidity might in fact be even smaller since perfusate within the liver contaminated the homogenate to a slight degree There was a difference between the measurements after a) ethanol and b) acid Thus it is seen from Fig 3 that for a given perfusate pH ethanol administration was accompanied by a lower homogenate pH than acid administration

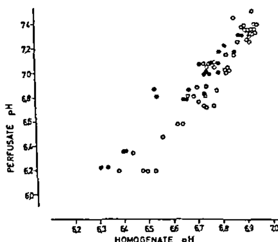


Fig 3 Relationship between perfusate pH and liver homogenate pH Each point represents the mean value obtained from two pH measurements in perfusate and four pH measurements in homogenate from one liver specimen The specimens were taken from 12 different livers 6 were perfused with ethanol (●) while acid was added to the perfusates of the other 6 (O)

When perfusate pH was lower than 7 1 this difference was statistically significant (Wilcoxon test $a < 0\ 1$) tested by comparing results within intervals of 0 1 or 0 2 perfusate pH units Since there were some variations between livers with respect to homogenate pH special experiments were performed to show that small reductions of homogenate pH due to ethanol could be found at even higher perfusate pH Five livers were perfused for 30 min without ethanol ethanol was then added and the perfusion continued for another 30 min Perfusate pH was fixed at 7 40 during the whole perfusion Homogenate pH was measured in three specimens taken before and in three taken after the addition of ethanol A reduction of homogenate pH was found after ethanol i all specimens in every liver The mean reduction was 0 04 pH units in three livers and 0 03 and 0 05 in the two others

Discussion

The isolated liver from fasted rats liberated acid to the perfusate This liberation as well as the acid production was most marked in the beginning of the perfusion This could reflect an adaption to the perfused system as the perfusate (blood diluted 1 3) contained approximately one third the amount of acid metabolites normally *present in blood The liver would thus act as a regulator of metabolite concentrations* in the perfusate (Schimassek 1963)

The production of acid was also dependent on perfusate pH Reduced acid production was the result of reduced perfusate pH The mechanism of acid liberation from the liver functioned however even at perfusate pH as low as 6 7 (Table I) The reduced release of acid metabolites from the liver at low pH and the increased production at high pH might contribute to the regulation of the acid base balance in blood It seems however more important that changes in blood pH would influence liver metabolism to produce more or less acid metabolites Observation indicating

reduced production of lactic acid pyruvic acid (Lueck and Miller 1970, Seglen 1972, Morland *et al*, to be published) and of keto acids (Morland *et al* to be published) at reduced pH support the results of our experiments

We measured a considerable increase in acid production in the perfused system after ethanol (Table I and II) This acid production seemed nearly constant (approximately 80 μmoles/h g liver) during the perfusion when perfusate pH was kept physiological (Fig 2) The acid release after ethanol could take place at perfusate pH as low as 6 2 The pH regulating principle discussed above could thus not compensate for the increase of acid production in the livers after ethanol. Ethanol is mainly oxidized in the liver by alcohol dehydrogenase and acetaldehyde dehydrogenase with NAD acting as coenzym in both reactions A constant metabolism of ethanol is accompanied by increased NADH/NAD ratio (for ref see Forsander 1970) and thus by increased H ion production Free acid is usually thought to be buffered at once although the existence of intracellular pH gradients have been proposed (for discussion, see Forsander 1970) A surplus of acid thus exists within the liver cells and this acid may be released to the blood as shown in our perfusion experiments Others have also found reduced pH in blood increased blood lactate (Himwick *et al* 1933 Nicholson and Taylor 1938 Krebs *et al* 1969) and increased acid production in livers (Seligson *et al* 1953 Tygstrup *et al* 1965 Kaden *et al* 1969 Morland *et al* 1972) after ethanol In experiments on perfused livers to which both ethanol (40 mM) and lactate (5 mM) was added (Morland *et al* unpublished) increase in lactate concentration could roughly account for only 25 per cent of the acid production Obviously other acids are of greater importance for the ethanol stimulated acid production

We found specially at low perfusate pH that liver homogenate pH was more reduced when the H ion had an intracellular origin (after ethanol) compared to experiments in which H ions entered the perfusate outside the liver (administration of HCl) (Fig 3) The pH gradient across the liver cell membrane was in this way dependent on the site of origin of the added H ions If intracellular pH gradients also existed pH in the compartments of ethanol oxidation would be lower than whole liver homogenate pH Thus one could assume metabolic important increases in acidity to occur in certain liver cell comparments after ethanol accompanied by only slight alterations in whole liver homogenate and blood pH If so pH sensitive intracellular reactions could be influenced by the metabolism of ethanol at physiological pH values

References

Forsander O A Influence of ethanol on the redox state of the liver *Quart J Stud Alcohol* 1970 *31* 550—570

Hermansen L and J B Osnes Blood and muscle pH after maximal exercise in man *J appl Physiol* 1972 *32* 304—308

Himwich H E L H Nahum N Rakieten J F Fazikas D DuBois and E F Gildea The metabolism of alcohol *J Amer med Ass* 1933 *100* 651—654

Kaden M N W Oakley and J B Field Effect of alcohol on gluconeogenesis using the isolated rat liver perfusion technique *Amer J Physiol* 1969 *216* 756—763

KREBS H A D J C CUNNINGHAM M STUBBS and D J A. JENKINS Effect of ethanol on postexercise lactacidemia. *Israel J med Sci* 1969 *5* 959—962

LUECK J D and L L. MILLER, The effect of perfusate pH on glutamine metabolism in the isolated perfused rat liver *J biol Chem* 1970 *245* 5491—5497

MORLAND J Chlorpromazine inhibition of tryptophan oxygenase *Biochem Pharmacol* 1971 *20* 547—552

MORLAND J T CHRISTOFFERSEN J B OSNES P O SEGLEN and K F JERVELL An effect of ethanol administration on tryptophan oxygenase in the perfused rat liver Decreased enzyme synthesis dependent on low perfusate pH. *Biochem Pharmacol* 1972 *21* 1849—1859

NICHOLSON W M and H M TAYLOR, The effect of alcohol on the water and electrolyte balance in man. *J clin Invest* 1938 *17* 279—285

SCHIMASSEK H Der Einfluss der Leber auf den extracellularen Redox Quotienten Lactat/Pyruvat Versuche an der isoliert perfundierten Rattenleber *Biochem Z* 1963 *336* 468—472

SEGLEN P O The effect of perfusate pH on respiration and glycolysis in the isolated perfused rat liver perfused with an erythrocyte—and protein free medium *Biochim biophys Acta* (Amst) 1972 *264* 398—410

SEGLEN P O and K F JERVELL A simple perfusion technique applied to glucocorticoid regulation of tryptophan oxygenase turnover and bile production in the isolated rat liver *Hoppe-Seyler's Z physiol Chem 1969 350 308—316*

SELIGSON D S S WALDSTEIN B GIGES W H MERONEY and V M SBOROV Some metabolic effects of ethanol in humans *Clin Res Proc* 1953 *1* 86

TYGSTRUP N K WINKLER and F LUNDQUIST The mechanism of the fructose effect on the ethanol metabolism of the human liver *J clin Invest* 1965 *44* 817—830

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Ventilation at Transition from Rest to Exercise

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Abstract

ASMUSSEN E *Ventilation at transition from rest to exercise* Acta physiol scand 1973 *89* 68—78

The fast neurogenic component of the increase in ventilation taking place at the transition from rest to exercise was studied by a breath to-breath analysis of $\dot{V}_E$ and P_{ACO_2} The questions investigated were 1) Is the fast neurogenic component correlated to the intensity of the exercise? 2) Is it due to a specific work stimulus to the respiratory center or caused by an increased sensitivity to existing chemical stimuli? 3) Is it related to movements or to the mechanical tensions in the working muscles? The results showed 1) There is a rough correlation between the fast neurogenic component and the power of the bicycle work performed 2) If the chemical stimuli expressed by P_{ACO} and P_{AO_2} are eliminated by voluntary hyperventilation in pure oxygen the fast neurogenic component disappears With one or both of these stimuli present exercise augments their effect 3) Transition from rest to static exercise (no movements) is not accompanied by a fast neurogenic component Rhythmic movements cause a rapid increase in $\dot{V}_E$ related to the frequency of movement Sudden loading of the bicycle during no-load pedalling causes a relatively slower further increase in ventilation apparently independent of the force exerted on the pedals but related to the power of work It is suggested that some metabolic factor in the working muscles is responsible for this part of the fast neurogenic component

Krogh and Lindhard (1913) showed that at the transition from rest to exercise on a bicycle ergometer the ventilation $\dot{V}_E$ increased suddenly—so rapidly that changes in the chemical composition of the blood could not be the cause for the increase They concluded that the fast changes must be due to factors of nervous origin and suggested irradiation to the respiratory center of the cortical motor impulses as the most likely explanation In subsequent experiments (1917) with electrically induced work, they also found a sudden increase in $\dot{V}_E$ although less abrupt and concluded that this must have been of reflex origin Likewise in the same articles they described the changes in $\dot{V}_E$ that occurred when in voluntary exercise the load on the bicycle was suddenly increased without the subject s knowledge Also here the fast changes were supposed to be of reflex origin

Key words ' Fast neurogenic component transition from rest to work transition from rest to static exercise rate of movement and fast neurogenic component work power and fast neurogenic component apnea and transition from rest to exercise sensitivity of respiratory center

This fast component in respiratory adaptation was studied again by *Asmussen and Nielsen* (1948) both in voluntary exercise and in electrically induced exercise *Krogh and Lindhard's* results were confirmed and it was further found that the magnitude of the rapid increase in V_E was systematically related to the severity of the exercise Reflexes originating in the exercising limbs were supposed to be the direct cause of the increase in V_E

These reflexes and their possible origin have been the object of many studies on humans (for references see *Dejours* 1959 1964 1967) but also in animal preparations (*Kao* 1963) and in exercising animals (*e g Flandrois et al* 1971) Although it is generally agreed that such reflexes exist their receptors — *i e* mechano-receptors chemo-receptors thermo-receptors or other — have not been definitely identified Their afferent pathways however have been located to the lateral columns of the spinal chord (*Kao* (1964)), and to small unmyelinated and myelinated fibres in the dorsal roots (*Mitchell and McCloskey* 1972)

The existence of a fast neurogenic component in the early adaptation of V_E to exercise does not necessarily mean that it is caused by a regulatory mechanism It might also be looked upon as a general disturbance in the otherwise chemically regulated V_E This point of view was taken by *Åstrand and Christensen* (1963) based on measurements of V_E at transition from rest to exercise of varying intensity They failed to observe a systematic relationship between the initial increase in V_E and the work intensity More recently *Åstrand and Rodahl* (1970) state as their opinion that the neurogenic factors cannot be considered as a regulating stimulus but act as an activator

In the experiments to be presented here it was intended to re investigate the fast neurogenic factor operating at the transition from rest to exercise in normal humans Three questions were investigated 1) Is the fast neurogenic component graded in relation to the work intensity? 2) Is the fast neurogenic component due to a specific respiratory stimulus or to a sensitizer (gain controller set point adjuster) for the respiratory center? 3) Is it related to *movement* or to *mechanical load* (force or power) in exercise?

Methods

V_E was registered breath to-breath by means of a 100 l accurately balanced spirometer fitted with a potentiometer in such a way that the movements of the spirometer were recorded as a staircase line on paper moving at known speed (67 5 mm s) Each step was measured for height (volume) with an accuracy of $\pm$0 025 l and length (time) and converted to ventilation per minute BTPS or ATPS Heart beats could be recorded on the same strip by means of an electrocardiograph and electrodes fastened to the chest of the subject Exercise was performed on a *Krogh* bicycle ergometer modified for sitting exercise by means of a specially built chair The subject worked in beat with a metronome and the load on the bicycle could be changed and switched on and off without the subject's knowing The composition of the inspired air could be altered *e g* by adding CO_2-enriched air to an inspiratory Douglas bag through a flow meter In this way the end tidal P_{CO_2} which was monitored continuously by an infrared CO_2 analyzer could be maintained fairly constant also in the transitory situation from rest to exercise In some experiments air mixtures with high CO_2 content (5) or low O_2 concentration (11) were inspired from the inspiratory bag In other experiments the subject hyperventilated voluntarily by breathing deeply in and out following a metronome at an individually convenient rhythm Hyperventilation was performed for periods of 3 min the last half minute and the ensuing period of apnoea or

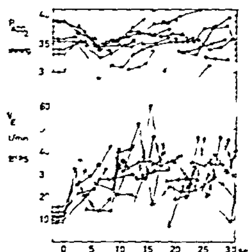


Fig 1 Breath-to-breath determination of $\dot{V}_E$ (bottom) and P_{ACO_2} (top) at transition from rest to exercise at 769 kpm min. 8 individual experiments

Fig 2. $\dot{V}_E$ and P_{ACO_2}, determined breath-to-breath and averaged from 8 to 10 single experiments each, on transition from rest to work at 0, 768, 1155 and 1920 kpm min. ±1 S.E. indicated. It was attempted to keep P_{ACO_2} constant by addition of CO to inspired gas

spontaneous breathing with 100 O in the inspiratory bag. End tidal P_{CO_2} after a 3 min period of hyperventilation was 8 to 12 mm Hg. In a few cases, as a check, the arterial P_{CO} was determined simultaneously on arterialized capillary blood from a fingertip using a Radiometer P_{CO} electrode. The results corroborated the readings on the P_{CO} analyzer.

Six young male students were subjects in the study. They were unaware of the purpose of the experiments and of the effect of hyperventilation etc. on respiration. The experiments were performed in the morning and the subject rested for at least 1/2 h in the chair of the ergometer before the actual experiments were begun. After this the subject was fitted with noseclip and mouthpiece and began breathing through a low-resistance respiratory valve turned to the atmosphere. After about 5 min, connection to the spirometer was established and recording began. When sufficient time had elapsed for a representative rest period to be recorded a metronome was started and the subject was asked to begin exercise immediately. No actual command was used, but the moment when the first movement was made was recorded on the registering paper. After 20 s of exercise work was interrupted, and a new rest period of 5 to 15 min began. Usually 4–6 expts. with or without voluntary hyperventilation, with different loads and various air mixtures, were made per session. The heart rate and the recorded endtidal P_{CO_2} served as signals for ensuring that recovery was complete between experiments.

As it will appear from the following, e.g. Fig. 1, the $\dot{V}_E$ scatters widely from breath to breath. Only when several experiments are averaged a clearcut result comes out. The averaging was performed in 2 ways. In the first series the breathing frequencies were so regular from experiment to experiment, that all first, second, third etc. breaths could be averaged and SE calculated. In the later series this was not the case so here all values of $\dot{V}_E$ within the same periods of time 2.5 or 5 s long were averaged and plotted against the average absolute time of the midpoints of the respective breaths. As each point thus becomes the average of a changing number of single values, from 3–4 up to 10–12, no attempts at calculating SE were made, but the points were connected by straight lines or a smoothed curve was drawn through them by eye for presentation in the figures.

Results

*1 Transition from rest to exercise of various intensities**

These experiments were performed on two subjects that reacted almost alike. Only data from one (P.D.M.) will be presented. The subjects started exercise with th

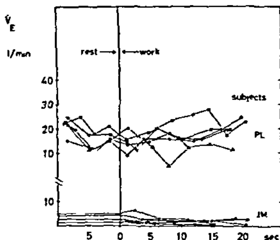


Fig 3 $\dot{V}_E$ at transition from rest to exercise after voluntary hyperventilation Different signatures denote 0 720 1080 1440 and 1800 kpm/min. Subject JM below PL above Each curve is averaged from 6 to 10 single experiments

following loads 0 768 1155 1920 kpm/min all at 60 rpm At each load 8—10 single experiments were made randomly varied and without the subject having knowledge of what the load would be

Fig 1 at 768 kpm/min *illustrates how much the data from individual experiments* scatter Even though attempts were made by adding CO_2 to the inspired air to prevent an initial drop in P_{CO} it nevertheless nearly always showed a fall As the respiratory frequency invariably tended to adjust to the beat of the metronome the temporal scatter of the single breaths was small enough to allow mean values of $\dot{V}_E$ from each single breath to be calculated In Fig 2 are presented the average $\dot{V}_E$-curves for work intensities between 0 and 1920 kpm/min together with the corresponding PA_{CO_2} values $\dot{V}_E$ shows a sudden increase at transition from rest to exercise with a tendency later to level off Except for the first breath $\dot{V}_E$ is the higher the higher the work load The PA_{CO_2}-curves show a slightly delayed dip of about 3 mmHg without any systematic correlation to the work intensity The levelling off of the $\dot{V}_E$-curves between 10 and 20—30 s has been noticed before (*e g Dejours* 1963) and is followed by a secondary rise to the true steady state level of $\dot{V}_E$ The corresponding results from the other subjects were qualitatively similar the only difference being that the $\dot{V}_E$-levels for the heavier work loads were somewhat lower Generally these results are not different from earlier data (*Asmussen and Nielsen* 1948) and show that the fast neurogenic component is roughly correlated to the intensity of the bicycle work.

2 The question whether the fast neurogenic component is due to a specific ventilatory stimulus or whether it is due to a sensitizing of the respiratory center was investigated by having the subjects hyperventilate voluntarily for 3 min the last half minute in pure oxygen By these means it was assumed that the normal chemical stimuli as represented by the arterial P_{CO_2} and P_{O_2} would be eliminated or at

* This series was performed by *S Davidsen* as part of his final exam for the cand scient degree

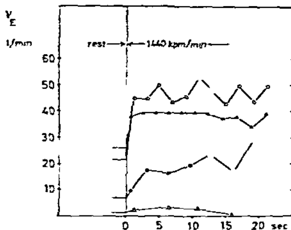


Fig 4 $\dot{V}_E$ at transition from rest to exercise at 1440 kpm/min. Subject JM. From below after voluntary hyperventilation breathing 11 % O_2 in nitrogen 4 % CO_2, and 11 % O_2 in nitrogen 5 % CO_2 in air Each curve averaged from 3 to 6 single experiments.

least greatly reduced Of the four subjects used for these experiments only one (J M) went into a true apnoea after the hyperventilation It lasted for more than 30 s and was then gradually disappearing as the P_{CO_2} built up In the experiments work was started after about 15 s of apnea at 60 rpm, but with loads giving powers ranging between 0 and 1800 kpm min. The subjects did not know beforehand what the load would be

As Fig 3 lower part shows J M s $\dot{V}_E$ changed very little or not at all at the transition from rest to exercise independently of the work load (0 720 900 1080 1400 or 1800 kpm min) The other three subjects did not exhibit any real apnoeic period after stopping voluntary hyperventilation but rather a continued period of gradually decreasing residual hyperventilation During this period their alveolar P_{CO_2} was always less than 20 mmHg and so presumably below the threshold value for P_{aCO_2} which according to e g *Nielsen and Smith* (1951) is about 30 mmHg The point of interest here was that when during this period of hyperoxic hypocapnia exercise was started $\dot{V}_E$ did not show any sudden increase but continued its gradually decreasing trend Fig 3 upper part shows average curves with work loads between 0 kpm/min and 1800 kpm min for subject P L

For the fast neurogenic component exercise *per se* does not seem to be a very powerful ventilatory stimulus. In the presence of existing chemical stimuli—*e g* in hypercapnia, hypoxia or a combination of the two—the fast neurogenic component , however is very pronounced as seen in Fig 2 and 4 The fast neurogenic drive consequently should be looked upon rather as a sensitizer to the respiratory center making it more sensitive to incoming signals from both central and peripheral chemoreceptors.

3 As the sudden transition from rest to exercise involves various neurogenic influxes to the respiratory center experiments were performed in which these presumably were varied These experiments were a) transition from rest to static (isometric) effort, b) transition from rest to no-load exercise on the bicycle with 36 60 or 90 rpm, c) transition from no-load exercise at 3 different frequencies to real work

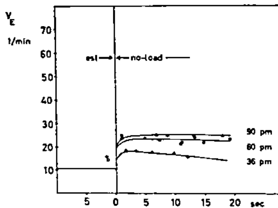


Fig 5 $\dot{V}_E$ at transition from rest to no-load exercise at 36 60 and 90 rpm Subject JS 11 % O_2 in inspired air Each curve average of 8 to 16 single experiments Curves drawn by eye

a) The subjects performed static work by pressing the feet hard against the pedals of the bicycle without movement $\dot{V}_E$ was measured breath by breath as usual both when the subject was breathing atmospheric air and when his ventilation was extra stimulated by CO_2 in the inspired air It was found that transition from rest to static effort did not cause any increase in $\dot{V}_E$ within the first 20 s of effort This is in accordance with what *Dejours* (1967) has noticed The heart rate however registered beat by beat during the same time increased from an average 57 beats/min to 72 beats/min within 5 s For the sudden increase in $\dot{V}_E$ accordingly movement seems to be essential

b) If the subject began pedalling with no load on the bicycle his $\dot{V}_E$ in all cases increased suddenly (*cf* Fig 2 0 kpm/min) If the subject had hyperventilated and was hypocapnic the increase if present at 60 rpm was small and transitory (lasting only about 5 s (*cf* Fig 3) If the subject was breathing 5 % CO_2 in air or 11 % O_2 in nitrogen the increase was larger and persistent The fast increase was dependent on the frequency of pedalling Fig 5 shows average curves with 36 60 or 90 rpm at transition from rest to no-load exercise when the subject was breathing 11 % O_2 in air Experiments with 5 % CO_2 in the inspired air showed the same relationship between pedalling rate and the fast response of $\dot{V}_E$ and even in the hypocapnic condition after hyperventilation this subject (J S) had a sudden increase in $\dot{V}_E$ at 90 rpm (but none or hardly any at 60 or 36 rpm)

c) If a subject was pedalling at a fixed frequency but with no load on the bicycle and the resistance on the bicycle then suddenly was switched on the $\dot{V}_E$ immediately began to increase not abruptly as at the transition from rest to movement but rather following a smooth curve If the load was so adjusted to the various pedalling frequencies that the power output became the same (here 1080 kpm/min) the effecs of different mechanical muscle tensions could be compared A series of experimentt with 11 % O_2 in the inspired air is averaged and presented in Fig 6 It can be seen that the no-load levels are the higher the higher the pedalling frequency and that the further increase in $\dot{V}_E$ on switching on the load is practically the same at all frequencies in spite of the differences in mechanical muscular tensions Similar results

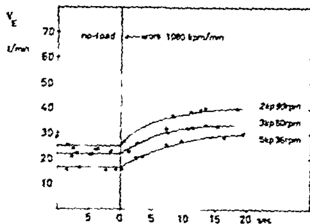


Fig 6 $\dot{V}_E$ at transition from no-load to exercise at 1080 kpm/min. 36, 60 and 90 rpm. Subject JS Inspired air 11 % O_2 Each curve drawn by eye average of 8–10 single experiments

were obtained with 5 % CO_2 in the inspired air (With hypocapnia after hyperventilation this subject had an increase in $\dot{V}_E$ with 36 rpm and a high load but none or hardly any at 60 and 90 rpm and the lower tensions) If the same load was put on at 60 rpm as at 36 rpm a much higher increase in $\dot{V}_E$ was observed corresponding to the greater power output 1800kpm/min vs 1080 kpm min)

All the increases in $\dot{V}_E$ measured at transition from no-load exercise to actual exercise began by the first or second breath after switching on the load and were pronounced already after 5–10 s i.e before any changes in the arterial blood to the respiratory center could have taken place and actually causing a decrease in P_{ACO_2}

Discussion

The present experiments have shown that the sudden increase in ventilation on transition from rest to exercise is graded in relation to the intensity of the exercise to the power This confirms previous findings of *Asmussen and Nielsen (1948)* and also those of *Krogh and Lindhard* from 1913 Several others have been unable to see any relation between the fast neurogenic component and work power (e g *Åstrand and Christensen (1964* *Flandrois et al (1971)*, who studied this problem in awake exercising dogs state that there is no relation between the work load and the fast component but their data e g their Fig 2 actually show that the initial increase is higher when their dogs run at 10 uphill than when they run at 5 or at the level— all at 10 km/h. Some authors e g *Paulev 1971* (have concentrated on the very first respiratory cycle after the signal to exercise has been given This is not necessarily the best way of studying the fast neurogenic component It is very possible that an anticipatory disturbance of the ventilation caused by the command or by moving the feet up to the pedals or on to the treadmill will mask a true neurogenic drive The second or third respiration after exercise has begun probably is less disturbed, and as it occurs within the first 5 to 10 s of exercise the chance that blood with increased P_{CO_2} or lowered P_{O_2} should have reached the chemoreceptors can be ruled

out During this period a lowering of the P_{ACO_2} is always seen In some of the present experiments it was attempted to keep the P_{ACO_2} constant by adding CO_2 to the inspiratory air at the transition from rest to exercise Fig 2 shows however that this was not achieved but at least the dip in the P_{ACO_2} was of the same size at all work levels and a possible damping effect of a low P_{ACO_2} on the ventilatory response was equalized The fast neurogenic component as depicted in Fig 2 for the first 5—15 s of exercise thus may be assumed to be practically the full rapid response to exercise at the different intensities

It has been postulated most recently by *Bea er and Wasserman* (1971) that the initial increase in V_E is a learned response and not a fundamental part of the control mechanism It can of course not be denied that subjects accustomed to start exercising at a given signal may respond with a more pronounced rapid increase—probably due to an anticipatory pre setting of the respiratory pattern But in the present experiments—as also in those of *Asmussen and Nielsen* (1948)—special care was taken to keep the subject dis informed as to the severity of the exercise Only when the exercise actually had begun did he sense the load and only then it was possible for him to adjust the ventilation to the work power A simple conditioned reflex thus can be ruled out as the fast neurogenic drive—signals from the moving and exercising limbs must be present The fact that also in electrically induced work a fast component is present (*Krogh and Lindhard* (1917) *Asmussen and Nielsen* (1948)) further speaks in favour of peripheral reflexes as being of predominant importance for the fast neurogenic component

The question whether the fast neurogenic drive is a respiratory stimulus by itself or whether it rather sensitizes the center towards the normal chemical stimuli was raised already by *Krogh and Lindhard* (1913) who came to the conclusion—mainly based on experiments with apnea in the transitory phase—that the mechanism by which the increased ventilation at the beginning of work is brought about is a sudden increase in the excitability of the respiratory centre The present experiments as exemplified by Fig 3 corroborate this conclusion The beginning of exercise within the wide range of work power from 0 to 1800 kpm/min seems to have no influence on the rise of V_E in the hypocapnic hyperoxic condition prevailing after 3 min of voluntary hyperventilation in pure oxygen It is however not justifiable to generalize from these examples because one of the four subjects used in this type of experiments did exhibit a fast graded response to exercise although smaller than when the chemical stimuli were present The only conclusion that can be drawn is that the fast neurogenic drive not necessarily in itself represents a stimulus to the respiratory motor centers

When chemical stimuli are present a pronounced effect is seen (Fig 4) It can also be seen that the comparatively weak stimulus of breathing 11 % O_2 is less augmented by exercise than the stronger stimulus of *e g* 5 % CO_2 in the inspired air This latter finding is at variance with the observations of *Cunningham et al* (1966) who found that V_E calculated from the very first breath after the beginning exercise was independent of the prevailing levels of P_{CO_2} and P_{O_2} They concl

that the neural stimulus does not immediately interact with the chemical stimuli. The reason for this discrepancy may lie in the fact that *Cunningham et al.* studied only the very first breath. As mentioned before, this quite possibly is so much influenced by anticipation that the true response becomes masked. Both *Krogh and Lindhard* (1913) and more recently *d'Angelo and Torelli* (1971) found that addition of CO_2 to the inspired air greatly increases the response to the "fast neurogenic drive". In the present experiments all subjects showed a large "fast neurogenic component" when the inspired air contained 5% CO_2, and a somewhat smaller one when its oxygen content had been lowered to 11% (*cf.* Fig. 4).

As mentioned before, the observation of *Dejours et al.* (1959) that in *static effort* no fast neurogenic component is present was confirmed. The "sense of effort" alone does not produce a fast increase in V_E—only dynamic exercise produces rapid ventilatory changes.

In dynamic exercise like bicycling there are two factors that combine to give the power of exercise—these are the frequency of the movements and the mechanical tension in the exercising muscles. The same power output (kpm/min) can be obtained, *e.g.*, at 36, 60 or 90 rpm on the bicycle if the loads are varied reciprocally, *i.e.*, in the ratio 5 to 3 to 2. With no load on the bicycle the effect of varying frequencies is shown in Fig. 5. Evidently rapid movements produce a larger "fast increase" than do slow movements. The increase in V_E are effected both by an increase in the respiratory frequency—from 17.8 breaths/min at 36 rpm to 19.3 at 60 rpm and 21.8 at 90 rpm—and by an increase in V_T. It is quite possible that in rhythm with leg movements bursts of activity in the γ loop are fed back to the central or peripheral respiratory motor centers in such a way that the respiratory stimuli produce a larger ventilation. *Eklund, von Euler and Rutkowski* (1966) found that stimuli like twisting the pinna of anesthetized cats increased the γ activity not only to the hind legs but also to the intercostal respiratory muscles. The "follow up *length* servo" activity of the γ loop to the moving legs thus quite likely is paralleled by an increased γ activity in the respiratory motor centers.

The possible importance of the muscle spindles in the exercising *muscles* for the fast neurogenic drive has been discussed by *Dejours* and his colleagues (*cf. Dejours* 1967). On the other hand it is also quite possible that joint receptors are directly involved as first *suggested* by *Comroe and Schmidt* (1943).

The effect of a sudden increase in load at different frequencies of pedalling is shown in Fig. 6 for the same subject as in Fig. 5. It shows that when the load is put on (by *operating a switch*), a second rise in V_E begins within 1 or 2 s, but the *increase* is gradual and continuous within the period of recording, 20 s. It will be noticed that the loads are different, varying reciprocally as the frequencies, so that the work intensity (*power*) becomes identical (1080 kpm/min) in the three series. The rapid onset of the rise indicates that it cannot be caused by changes in the blood that irrigates the central control mechanisms, including the arterial chemoreceptors—*in* fact PA_{CO_2} *decreased* with the rise in V_E. The receptors registering length changes in the muscles, *e.g.*, muscle spindles—are probably not stimulated differently before

and after the load was applied but receptors registering muscle tensions—*e g* the tendon organs and possibly also joint receptors must be quite differently stimulated by the high loads at 36 rpm as compared to the low loads at 90 rpm Nevertheless the changes in V_E are very much alike although occurring at different levels of ventilation The comparatively slow and gradual increase in V_E speaks against a mechanical reflex as the direct cause for the increase The power was equal in the three work series and presumably therefore also the energy liberation in the muscles One possible explanation would therefore be that local muscle receptors stimulated by metabolites produced in amounts corresponding to the work intensity and gradually building up to a steady state level could be the origin of this second neurogenic drive *Stegemann* (1963) has reported on the existence of such receptors in ischaemic human muscles and *Hnik* (1971) found evidence for their existence in rat muscles and suggested that increased extracellular K^+ might be the stimulus The fact that they do not operate in static effort (*cf* above) seems however to contradict this interpretation and so does the finding that immediate blocking of the blood flow through exercised muscles at the stop of exercise—which procedure presumably would trap any metabolites in the muscles—does not prevent the ventilation from decreasing corresponding to the decrease in V_{O_2} (*Asmussen and Nielsen* (1948) *Dejours* (1963)) Again it seems that movement is a necessary condition also for this effect to become manifest A possible explanation is that the effect of the metabolites is to increase the sensitivity of the mechanoreceptors towards their stimuli Such a suggestion was made by *Dejours* in 1967 The question of which metabolite(s) that may have this effect is open to speculation Lactic acid can probably be ruled out because of its relatively late appearance in the chain of metabolic processes in exercise but several others are possible besides K^+ as proposed by *Hnik* A hormone as nor adrenaline is known to be active in increasing the heart rate also in exercise In all the present experiments the heart rate increased within one or two seconds from the start of the exercise but this happened also under circumstances when no changes in V_E occurred *e g* after hyperventilation and with static effort (*cf* above) Thus the sudden increase in heart rate and in ventilation at the start of exercise appears to be governed by different factors and nor adrenaline is hardly responsible for the fast ventilatory response *Wigertz* (1971) found correspondingly in an analysis of the dynamics of the respiratory and circulatory adaptations to exercise that the two functions had quite different time constants

The relatively slow increase in ventilation that follows a sudden increase in mechanical load was present in the experiments with 11 % O_2 and in those with 5 % CO_2 in the inspired air After hyperventilation there also was a change at transition from no-load to loaded exercise but considerably less than in the other two conditions

In summary the present experiments have confirmed earlier reports on a fast neurogenic component in the ventilatory adaptation to exercise This component is roughly related to the intensity of the exercise It is probably not caused by an independent work stimulus but rather by a sensitizing effect on the respiratory

centre towards existing chemical stimuli The sensitizing effect is of peripheral reflex origin and presupposes movement It can be split up in an abrupt effect related to the movements per se and roughly graded after their frequency and a more gradual effect related to work power and thus to the local metabolic rate It is separated temporally from the subsequent stimulation of the V_E that occurs when blood from the working muscles reaches the central and arterial chemoreceptors usually 15 to 25 s after the start of exercise The fast neurogenic component seen at the start of dynamic exercise is the resultant of these two factors interacting with or adding to the effect of the prevalent respiratory stimuli

References

ASMUSSEN E and M NIELSEN Studies on the initial changes in respiration at the transition from rest to work and from work to rest *Acta physiol scand* 1948 *16* 170—285

ÅSTRAND P O and E H CHRISTENSEN Hyperpnoea of exercise In *The regulation of human respiration* Oxford Blackwell 1963 515—524

ÅSTRAND P O and K RODAHL In *Textbook of Work Physiology* New York McGraw Hill 1970 p 241

BEAVER, W L and K WASSERMAN Tidal volume and respiratory rate changes at start and end of exercise *J appl Physiol* 1970 *29* 872—876

COMROE J H and C F SCHMIDT Reflexes from the limbs as a factor in the hyperpnoea of muscular exercise *Amer J Physiol* 1943 *138* 536—547

CUNNINGHAM D J C B B LLOYD and D SPURR The relationship between the increase in breathing during the first respiratory cycle in exercise and the prevailing background of chemical stimulation *J Physiol* (Lond) 1966 *185* 73—74P

D ANGELO and G TORELLI Neural stimuli increasing respiration during different types of exercise *J appl Physiol* 1971 *30* 116—121

DEJOURS P La regulation d la ventilation au cours de l exercise musculaire chez l homme *J Phys ol* (Paris) 1959 *51* 163—261

DEJOURS P The regulation of breathing during muscular exercise in man A neuro-humeral theory In *The ulation of human respiration* Oxford Blackwell 1963 535—547

DEJOURS P Control of respiration in muscular exercise In *Handbook of Physiology* Sect on 3 Respiration Vol I 1964 631—648

DEJOURS P Neurogenic factors in the control of ventilat on during exercise In *Physiol gy of muscula exercise* Amer heart association monograph number 15 1967 146—153

EKLUND G C VON EULER and S RUTKOWSKI Spontaneous and reflex activity of intercostal gamma neurones *J physiol* (London 1964 *171* 139—163

FLANDROIS R J R LACOUR and H OSMAN Control of breathing in the exercising dog *R sp Physiol* 1971 *13* 361—371

HNIK P *Activation of muscl affe nts induced by mu le work* Satellite symposium on Physical Fitness Prague 1971 26

KAO F F An experimental study of the pathways involved in exercise hyperpnoea employing cross-circulation techniques In *The gulation f human resp ation* Oxford Blackwell 1963 461—507

KROGH A and J LINDHARD The regulat on of respiration and circulation during the initial stages of muscular work *J Physiol* (London 1913 *47* 112—136

KROGH A and J LINDHARD A compar son between voluntary and electrically induced muscular work in man *J Physiol* (London) 1917 *51* 182—201

MITCHELL J H and D J McCLOSKEY Reflex cardiovascular and respiratory responses originating in exercising muscle *Fed P oc* 1972 *31* 311

NIELSEN M and H SMITH Studies on the regulat on of resp ration in acute hypoxia *A ta physiol scand* 1951 *24* 293—313

PAULEY P E Respiratory and cardiac responses to exerc se in man *J appl Physiol* 1971 *30* 165—172

STEGEMANN J Zum Mechanismus der Pulsfrequenzeinstellung durch den Stoffwechsel I—IV *Pfl ers A ch ges Physiol* 1963 *276* 481—492

WIGERTZ O Dynamics of respiratory and circulatory adaptation to muscular exercise in man *Acta physiol scand* 1971 Suppl *363*

Acta physiol scand 1973 89 79—90

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Colonic Motility in the Cat

I Extraluminal Strain Gage Technique Influence of Anesthesia and Temperature

By

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Abstract

Rostad H *Colonic motility in the cat I Extraluminal strain gage technique Influence of anesthesia and temperature* Acta physiol scand 1973 *89* 79—90

A technique for recording colonic motility *in vivo* with extraluminal strain gage transducers is described This technique allowed segmental recording of the motility and proved more sensitive than intraluminal pressure devices The transducers did not appear to interfere with the spontaneous colonic activity Immediately after laparotomy considerable activity was observed in all parts of the colon The degree of spontaneous motility varied from animal to animal and from time to time in the same animal The colon was seldom quiet unless the anesthesia was deep Two main types of spontaneous contraction waves were observed (i) slow waves of 0 5—2 0 min duration and (ii) rhythmic waves of a frequency of 4—7 per min. Cooling of the abdominal wall resulted in augmented colonic motility warming caused relaxation and reduced activity Variation in body temperature between 36 and 39 did not influence the motility

The purpose of the present study was to develop an adequate technique for studying the extrinsic and central nervous control of colonic motility in the cat As one of the problems was to establish the border between parasympathetic sacral and vagal innervation of the colon the method must be capable of simultaneously recording the motility of various segments of the colon Further the recording method should be sensitive enough to reproduce relatively small changes in contractions and interfere minimally with the normal activity of the intestine Previous methods most commonly used do not satisfy these requirements

The method of direct observation of the serosal surface of the colon necessitates throughout the experiment an exteriorized organ or an open abdominal cavity with an organ bath arrangement (Barcroft and Steggerda 1932 Wang *et al* 1940) These conditions are unphysiological and inconvenient for the present purpose and no continuous records are obtained unless expensive movies are used Similar objections apply to the radiological methods earlier described (Cannon 1902 McFadden Loughridge and Milroy 1935 Ritchie 1968)

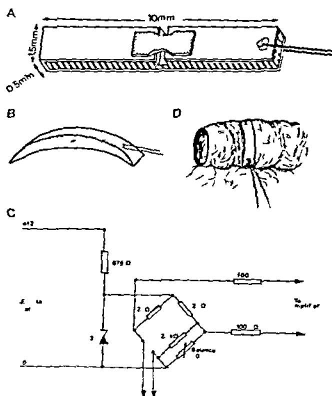


Fig 1 Extraluminal strain gage transducer *A* Pure transducer with leading wires soldered to the leading plates. *B* The insulated transducer embedded in a silicon rubber cast *C* Electrical bridge circuit. *D* The transducer sutured to the serosal surface of the colon

) Intraluminal pressure recordings by means of balloons or open ended tubes have been extensively used for the study of colonic motility both in animals and humans (Templeton and Lawson 1931 Garry 1933 Gray *et al* 1955 Connell 1961 Kock, Hulten and Leandoer 1968 Semba and Fujii 1970 a.o) However in a hollow tube like the colon where different segments may contract independently of each other it is impossible to be sure that the pressure changes recorded are really derived from the area of the recording tip (Painter 1968 Schuster and Mendeloff 1970) Moreover the method interferes with the normal motility of the colon Finally in longlasting experiments faecal masses adhering to the surface of the balloons or plugging of the open ended tubes prevents a true recording of the events

In recent years various forms of extraluminal force strain gage transducers have found their application in recording of gastrointestinal motility (Jacoby Bass and Bennett 1963 Bass and Wiley 1966 Reinke Rosenbaum and Bennett 1967) This technique meets most of the aforementioned criteria for recording However from the reports just mentioned it appears that the sensitivity of the strain gages used are insufficient for the present purposes

High sensitivity is provided by semiconductor strain gage transducers and these can also be given a small and suitable shape The present communication describes the use of such transducers for recording of colonic motility and compares this

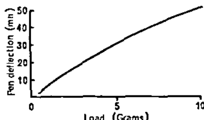


Fig 2 Calibration curve for extraluminal strain gage transducer

extraluminal method with intraluminal pressure recording Further it describe the pattern of colonic motility and some observations on the influence of laparotomy anesthesia temperature and a muscle relaxing drug on the motility These observa tions form the basis for subsequent reports

Methods

The recording device — The strain gage chosen was a Pixie transducer Model 8101 (Endevco Corporation, California) with good linearity and moderate and predictable temperature coefficients The semiconductor element is mounted on a small beam measuring 10×1.5×0 5 mm (Fig 1 A) Deflections of the beam cause the element to change its length which produces a proportional change in resistance of the element, modulating a passing current.

Thin lead wires were soldered to the leading plates of the beam The transducer was in sulated by several coats of varnish and casted in a semicircular form of Silicon rubber RTV-630 (Dow Corning Corp) (Fig 1 B) The transducer was connected with a Grass Model 7 polygraph through a balanced bridge circuit (Fig 1 C)

The transducers were fixed transversely to the surface of the colon with one silk suture in each end (Fig 1 D) In the initial experiments transducers with a rectangular form were also sutured to the colon in its longitudinal axis in order to monitor the longitudinal muscles of the bowel

Calibration of the transducers was achieved by fixing one end and mechanically loading the other with weights from 0 5 to 10 g Each transducer was found to be approximately linear within this range (Fig 2)

Material

The recording technique described has been utilized in 1?5 cats mostly male weighing *between 1 8 and 5 2 kg usually more than 3 kg The last 105 cats were also used in the stud* of the extrinsic and central nervous control of colonic motility The food intake of several cats was regulated for a m nimum of 2 weeks in order to bring their constipation under control During the last 5—6 days they were given only milk and subsequentl starved 24 h prior to the operation. Such procedures usually alleviated the constipation, though the colon often still contained semisolid or solid faeces As far as poss ble male cats were used Tl ese were easier to keep under a light and even level of anesthesia and ecording artifacts from accompanying contraction of the uterus in the females could be a oided

Anesthesia and operati e techniques

Initially the animals were anesthetized with an i p injection of penthobarbital sodium (Nembutal® Abbott) 30—40 mg/kg b w When neces ary small additional doses were administered i v during the experiment. In order o keep the animal under light anesthesia and to prevent deep abdominal respiratory movem nts and abdominal muscle contractions a neuromuscular blocking agent, pancuronium bromide Pa ulon® Organon) 0 —0 3 mg/kg b w was given i v and artific al respiration maintained During the experiment additional doses of 0 1—0 15 mg/kg b w were administered usuall every second or third hour Body temperature was kept as con tant as pos ble by means of heated pad be ow the animal Blood p essure was measured f om a femoral artery by a Statham pressure transducer and

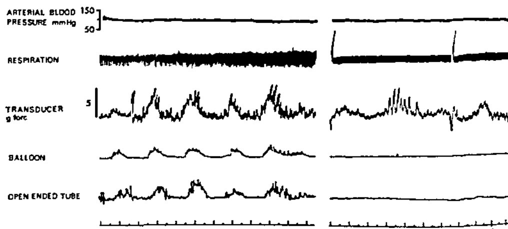


Fig 3 Cat 5 1 kg Motility in the proximal part (5 cm) of the colon recorded with extra luminal strain gage transducer intraluminal balloon and open ended tube Left side At the early stage of the experiment Right side 4 h later Note the loss of sensitivity of the intraluminal devices

transferred to the polygraph Thoracic respiratory movements were recorded through a Grass volumetric pressure transducer PT 5 A connected with the polygraph A femoral vein was cannulated for administration of anesthetics and other drugs and a tracheal cannula inserted to allow free airways and artificial respiration

The abdomen was opened through a midline incision and the small intestine extirpated as a potential source of recording artifacts This removal did not seem to interfere with the vascular or nervous supply of the colon For the same reason the bladder was placed outside the abdominal wall and drained and in the female cats the uterus was removed

3 Pixie transducers were sutured to the surface of the colon in various locations 5—7 cm apart one usually near the coecum one in the middle part of the colon and a third in the colon sigmoideum When intraluminal pressure was recorded by means of balloons or open ended tubes these were introduced into the colon via small antimesenteric incisions of the intestinal wall Tubes with inner diameter from 0 5 to 2 0 mm were used The balloons were filled with 0 25—1 0 ml water and the pressure recorded via Statham pressure transducers on the polygraph The open ended tubes were filled with saline or Tyrode solution and the intraluminal pressure recorded in the same way

Results

Comparison of the intraluminal and extraluminal methods In order to compare these two recording methods a balloon and an open ended tube were introduced close to each other into the lumen of the bowel in 10 of the initial experiments The Pixie transducer was fixed to the wall of the same segment

During the early stage of each experiment the intraluminal balloon and open ended tube methods appeared to give results similar to those of the extraluminal method although the latter was considerable more sensitive (Fig 3 left side) The higher sensitivity of the extraluminal method is essential in studies of the nervous influence of the colonic motility Moreover after a couple of hours the intraluminal methods were unsatisfactory because of minimal or no sensitivity (Fig 3 right side) This was probably due to embedding of the balloon in faecal masses and plugging of the

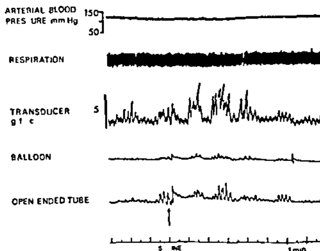


Fig 4 Cat 5 1 kg Motility in the proximal part (5 cm) of the colon recorded with an extraluminal strain gage transducer an intraluminal balloon and an open ended tube Note the augmentation of motility after infusion of 0 5 ml saline through the latter

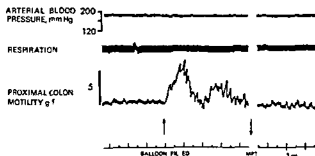


Fig 5 Cat 3 2 kg Colonic motility in the proximal part (5 cm) recorded with an extraluminal strain gage transducer Effect of filling and emptying (1 h later) of a small intraluminal balloon placed in the same segment (as the transducer)

open ended tubes The embedding was difficult to avoid The plugging of the tubes could be reduced by infusion of small quantities of fluid through the tubing However this procedure augmented the colonic activity recorded by the extra luminal transducer (Fig 4) The adhesion of intestinal contents to the surface of the balloon was also difficult to avoid

The intraluminal balloons were sometimes observed to induce some degree of colonic activity This could be shown by infusion of 0 5—1 0 ml water into the empty balloon and observation of the motility recorded by the extraluminal trans ducer After emptying the balloon the activity decreased again (Fig 5) No inter ference with the colonic activity could be observed after suture of the transducers to the wall of the colon

The extraluminal strain gage transducers described are more sensitive than intraluminal pressure devices Furthermore they do not appear to interfere with the spontaneous activity of the bowel

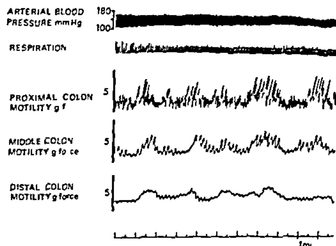


Fig 6 Cat 2 6 kg Colonic motility recorded with extra luminal strain gage transducer Note the difference of motility pattern in the proximal and distal part of the colon

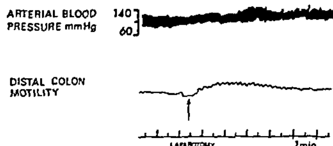


Fig 7 Cat 4 2 kg Effect of laparotomy on the distal colonic motility recorded by means of an intraluminal open ended tube introduced through the anus before the operation

Patterns of colonic motility in extraluminal recordings Two main types of spontaneous contraction waves were observed

(i) Slow waves of 0 5—2 0 min duration accompanying a force of 2 0—5 0 g contraction usually occurring at irregular intervals but sometimes showing a tendency towards rhythmicity (Fig 6) These slow waves usually appeared simultaneously in all segments of the colon but were most conspicuous in the distal part.

(ii) More rapid and rhythmic waves of 5—15 s duration appearing alone or superimposed on the slow waves The faster waves had a frequency of 4—7 per min and were of 0 5—2 0 g force They were most marked in the proximal portion of the colon where the frequency usually was higher than in the lower segments These waves were least susceptible to anesthesia and poor condition of the animal b t under such circumstances the frequency usually decreased

The degree of spontaneous motility varied from one animal to the other and from time to time in the same animal However the colon was seldom quiet unless the anesthesia was very deep or the animal s condition was poor

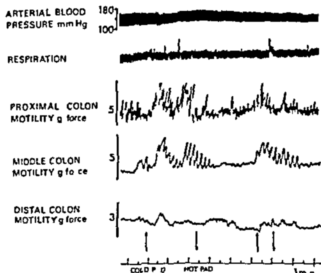


Fig 8 Cat 2 6 kg Effect on the colonic motility of cold and warm pad to the surface of the abdominal wall Note the immediate change in motility as a result of the application of the pad

Throughout the experiment periods of activity ordinarily alternated with periods of relative inactivity but complete silence only exceptionally occurred On the other hand strong sustained contractions transmitted down the colon were seen infrequently These were probably associated with propulsive activity

In the initial experiments an attempt was made to separate the longitudinal and circular muscle contractile activity by way of one transducer in the longitudinal and one in the transverse axis However aside from minimal quantitative differences the spontaneous contractile activity pattern observed in the two transducers was usually almost the same

Effects of laparotomy In 10 experiments three extraluminal transducers were attached to various parts of the colon and the motility was studied

Immediately after laparotomy when the gut was exposed to air and subjected to manipulations considerable activity was usually observed in all parts of the colon Occasionally longitudinal contractions were so vigorous that the whole organ shortened considerably The effect of laparotomy *per se* on the motility was tested in 9 experiments by intraluminal pressure recording via an open ended tube introduced into the colon through the anus before the operation The typical result of an experiment is shown in Fig 7 namely a rise in the intraluminal pressure and an augmentation of rhythmic contractions when the peritoneal cavity was opened Abundant activity was also present after the end of the operation When the animals had been left undisturbed for 20—30 min the peristaltic activity usually decreased In the course of 1—2 hours there was again some augmentation though usually not to the same degree as present immediately after the operation

Influence of anesthesia and muscle relaxing drug In 12 experiments the effect of anesthesia without muscle relaxing agent present was assessed To avoid artifacts

because of muscular rigidity the anesthesia was deepened by intravenous administration of additional doses of penthobarbital sodium This invariably reduced and sometimes abolished the colonic motility as well as the responses to peripheral and central nervous stimulation This was also the case when chloralose was used as the anesthetic Thus studies of the nervous control of the motility requires a light level of anesthesia at least with respect to the two anesthetics tested To obtain relaxation of the abdominal wall the anesthesia has to be combined with a muscle relaxing agent

Therefore the possible influence of the muscle relaxing agent pancuronium bromide on the motility was tested in another series of experiments The drug was not administered until the colonic motility was stabilized at least 1 1/2—2 h after the operation A dose of 0 2—0 3 mg/kg b w was usually required to cause respiratory arrest This did not reduce the colonic activity On the contrary, sometimes the motility increased presumably secondary to the elimination of the muscular rigidity present before the injection The activity in relatively deep anesthesia was unaffected by pancuronium bromide The effect on the blood pressure and heart rate was negligible Only exceptionally a slight transient fall in pressure was observed

Influence of temperature changes Application of a cold pad (4—5° C) to the surface of the abdominal wall resulted in augmented colonic motility This gradually subsided after 3—5 min A warm pad (40—50° C) caused relaxation and reduced peristaltic activity for a couple of minutes (Fig 8) This probably explains the well known clinical observation that application of hot pads to the abdominal wall alleviates abdominal pains

Variations in body temperature between 36 and 39° C during the experiment did not appear to influence the colonic motility When the temperature occasionally fell to 35° C or lower the activity decreased However this was usually only the case when the animal s condition was poor towards the end of longlasting experiments

Discussion

Methodological considerations

In the introduction the difficulties in obtaining a reliable recording of colonic motility were discussed In the present experiments the extraluminal strain gage transducers have been shown to be better suited for the present purpose than intraluminal pressure recording for three reasons

(1) The sensitivity of the extraluminal method is superior to the intraluminal one especially in longlasting experiments The intraluminal pressure changes have to be transmitted along fluid filled tubes which might distort the responses The problem of blocking the open ended tube by faecal masses or mucosal folds has partly been solved by the use of several small terminal holes in the tube and by

frequent infusion of fluid in small quantities through the tubing (Quigley and Brody 1952 Davidson *et al* 1956 Truelove 1966) This procedure however, commonly produced artifacts in the present experiments The same experience was reported by Edwards (1965) Complete removal of all contents in the colon and filling the lumen with fluid (Hulten 1969) reduces the problem with plugging of the terminal holes but fails to prevent the loss of sensitivity due to volume changes transmitted along fluid filled tubes

(ii) The replacement of solid or semi solid faecal masses by fluid is an un physiological condition and might interfere with the spontaneous colonic motility Similar objections may be raised with regard to the intraluminal balloon which acts as a foreign body (Chaudhary and Truelove 1961) and when distended may induce activity as shown in the present experiments When the segment overlying the balloon contracts the balloon remains in that segment and thus may offer a greater resistance than that encountered under normal conditions when the contents in the bowel are free to shift into another segment (Truelove 1966) In this way the normal passage of the intestinal contents will be disturbed

(iii) The extraluminal strain gage transducers have the advantage of recording the movements at various segments of the colonic wall directly Intraluminal pressure recordings with balloons or open ended tubes reflect only the result of the contractile activity of the muscles of the gut in an indefinite area Volume recording depends on investigation of the whole fluid filled colon or isolated parts of it as a unit This implies that volume changes provoked by contraction in one segment of the gut may be neutralized by simultaneous relaxation in another

A possible source of error when using extraluminal strain gage transducers is that a contraction of segments not implanted with transducers could be missed and on the contrary give rise to a false recording of relaxation because of passive increase in the circumference of the colonic wall under the transducer (Jacoby Bass and Bennett 1963) However this did not occur when transducers were implanted near each other and compared with other methods

Although the extraluminal transducers in the initial experiments were sutured to the bowel both in the transverse and longitudinal axis a satisfactory separation of the muscle contractile activity in the longitudinal and circular layer was not obtained Similar experience was reported by Reinke Rosenbaum and Bennett (1967)

Peristaltic pattern

The two main types of peristaltic waves obtained by extraluminal strain gage recording in the present experiments seem to be equivalent to 2 of the 3 types of waves described by Templeton and Lawson (1931) who used intraluminal balloons in the proximal and distal colon of dogs Their type 1 waves are similar to the more rapid rhythmic waves most marked in the proximal colon while the slow waves with the rhythmic waves surmounted most likely correspond to the type 2 of Templeton and Lawson Their type 3 were waves of still longer duration than type

2 However, later authors using various forms of intraluminal pressure recording have not found this classification satisfactory because of the very irregular patterns of activity often observed (Davidson *et al* 1956 Chaudhary and Truelove 1961, Connell 1961 Ritchie Ardran and Truelove 1962 Connell Texter and Vantrappen 1965 Deller and Wangel 1965 Bloom Lopresti and Farrar 1968) Nevertheless in the present experiments with extraluminal strain gage recording two characteristic types of contraction waves were regularly obtained

Although great variations in the normal motility pattern were observed in the present experiments a significant degree of activity was always present On the other hand authors using the intraluminal balloon technique found periods of complete or almost complete colonic inactivity in animal experiments both in the anesthetized and conscious state (Garry 1933 Gray *et al* 1955 Hukuhara and Miyake 1959) Investigations of human colonic motility utilizing balloons or open ended tubes have revealed activity for only 15—60 % of the time of recording (Adler Atkinson and Ivy 1941 Spriggs *et al* 1951 Davidson *et al* 1956 Fink and Friedman 1960 Connell 1961) The reports of quiet periods have probably been due to the insufficient sensitivity of the recording technique utilized

Effects of laparotomy

Increased motility as an effect of laparotomy was also observed by Edvardsen (1968) concerning the motility of the bladder while Hulten (1969) described no clearcut motility in any part of the colon at the early stage after laparotomy urgical trauma is known to depress gastrointestinal motility because of reflex activation of adrenergic fibres (for references se Jansson 1969) The mechanisms behind the temporary increased motility immediately after laparotomy are not fully understood

Influence of anesthesia

Deep anesthesia was associated with decreased peristaltic motility and minimal or negligible effects of nerve stimulation Therefore a light anesthesia is essential in such experiments In lightly anesthetized animals however artifacts readily occurred because of the relatively high sensitivity of the extraluminal strain gage transducers A muscle relaxing agent was therefore required Pancuronium bromide is five times more potent than d tubocurarine (Sellick 1970) Furthermore the histamine releasing effect and ganglion blocking action is much less (Buckett *et al* 1968 Sellick 1970) The effect on the circulation is minimal (Baird and Reid 1967 Crul 1970) By means of this drug and artificial respiration the dose of anesthetics could be kept at a minimal level without recording artifacts

Influence of temperature changes

The influence of hot and cold pads applied to the abdominal wall on the colonic motility was studied by Grace Wolf and Wolff (1951 pp 116—136) in an unanesthetized patient with a colostomy Relaxation of the colon was noted im

mediately when a water bottle of 50° C was placed on the abdomen The changes were approximately identical with those observed in the pre ent series These results were not due to strain gage artifacts because temperature changes in the transducer produced the opposite effect i e increased temperature simulated colonic contrac tion and vice versa Therefore changes of colonic motility as a result of hot and cold pads to the abdominal wall were due to real tonal and motility changes in the bowel Small variations of the body temperature did not appear to influence the colonic motility

References

ADLER H F A. J ATKINSON and A C IVY A study of the motility of the human colon An explanation of dysenergia of the colon or of the "unstable colon *Amer J dig Dis* 1941 *8* 197—202

BAIRD W L. M and A M REID The neuromuscular blocking properties of a new steroid compound pancuronium bromide *Brit J Anaesth* 1967 *39* 775—779

BARCROFT J and F R STEGGERDA Observations on the proximal portion of the exteriorized colon *J Physiol* (Lond) 1932 *76* 460—471

BASS P and J N WILEY Electrical and extraluminal contractile force activity of the duodenum of the dog *Amer J dig Dis* 1966 *10* 183—200

BLOOM A A P LOPRESTI and J T FARRAR Motility in the intact human colon *Gastroenterology* 1968 *54* 232—240

BUCKETT W R. C E B MARJORIBANKS F A MARWICK and M B MORTON The pharmacology of pancuronium bromide (Org NA97) a new potent steroidal neuromuscular blocking agent. *Brit J Pharmacol* 1968 *32* 671—682

CANNON W B The movements of the intestines studied by means of the rontgen rays *Amer J Physiol* 1902 *6* 251—277

CHAUDHARY N A and S C TRUELOVE Colonic motility a critical review of methods and results *Amer J Med* 1961 *31* 86—106

CONNELL A. M The motility of the pelvic colon. *Gut* 1961 *2* 175—186

CONNELL A M E C TEXTER and G VANTRAPPEN Classification and interpretation of motility records *Amer J dig Dis* 1965 *10* 481—483

CRUL, J F Studies on new steroid relaxants *Excerpta med (Amst) Int Congr Ser 200* 1970 418—424

DAVIDSON M M H SLEISENGER, T P ALMY and S Z LEVINE Studies of distal colonic motility in children *Pediatrics* 1956 *17* 807—819

DELLER, D J and A G WANGEL Intestinal motility in man *Gastroenterology* 1965 *48* 45—57

EDVARDSEN P Nervous control of urinary bladder in cats *Acta physiol scand* 1968 *72* 157—171

EDWARDS D A. W Techniques and interpretations of measurement of gastro-intestinal pressures *Ann roy Coll Surg Engl* 1965 *37* 275—284

FINK S and G FRIEDMAN The differential effect of drugs on the proximal and distal colon *Amer J Med* 1960 *28* 534—540

GARRY R C The nervous control of the caudal region of the large bowel in the cat *J Physiol* (Lond) 1933 *77* 422—431

GRACE W J S WOLF and H G WOLFF *The human colo* New York P B Hoeber 1951 239 pp

GRAY G W L C HENDERSHOT R M WHITROCK and M H SEEVERS Influence of the parasympathetic nerves and their relation to the action of at opin n the leum and colon of the dog *Amer J Physiol* 1955 *181* 679—687

HUKUHARA T and T MIYAKE The intrinsic reflexes in the colon *Jap J Physiol* 1959 *9* 49—55

HULTEN L. E trinsic nervous control of colonic motility and blood flow *Acta physiol scand* 1969 Suppl 335

JACOBY H I P BASS and D R. BENNETT In vi o extraluminal contractile fo ce transducer for gastrointestinal muscle *J appl Physiol* 1963 *18* 658—665

JANSSON G Extrins c nervou control of gastric motilit *Acta phy ol s and* 1969 Suppl 326

KOCK, N G L HULTEN and L LEANDOER, A study of the motility in different parts of the human colon *Scand J Gastroent* 1968 *3* 163—169
McFADDEN G D F J S LOUGHRIDGE and T H MILROY The nerve control of the distal colon. *Quart J exp Physiol* 1935 *25* 315—327
PAINTER, N S The correlation of the pressures in the human colon with the shape of the colonic lumen as shown by cineradiography combined with simultaneous pressure recording *Amer J dig Dis* 1968 *13* 468—483
QUIGLEY J P and D A. BRODY A physiologic and clinical consideration of the pressures developed in the digestive tract. *Amer J Med* 1952 *13* 73—81
REINKE D A A H ROSENBAUM and D R. BENNETT Patterns of dog gastrointestinal contractile activity monitored in vivo with extraluminal force transducers *Amer J dig Dis* 1967 *12* 115—141
RITCHIE J A Colonic motor activity and bowel function *Gut* 1968 *9* 442—456
RITCHIE J A G M ARDRAN and S C TRUELOVE Motor activity of the sigmoid colon of humans *Gastroenterology* 1962 *43* 642—668
SCHUSTER, M M and A I MENDELOFF Characteristics of rectosigmoid motor function Their relationship to continence defecation and disease *Progr Gastroent* 1970 *2* 200—220
SELLICK B A Pancuronium bromide Clinical experience of a new muscle relaxant *Excerpta med (Amst) Int Congr Ser 200* 1970 1144—1147
SEMBA T and Y FUJII Relationship between venous flow and colonic peristalsis *Jap J Physiol* 1970 *20* 408—416
SPRIGGS E A C F CODE J A. BARGEN R. K. CURTISS and N C HIGHTOWER, Motility of the pelvic colon and rectum of normal persons and patients with ulcerative colitis. *Gastroenterology* 1951 *19* 480—491
TEMPLETON R. D and H LAWSON Studies in the motor activity of the large intestine *Amer J Physiol* 1931 *96* 667—676
TRUELOVE S C Movements of the large intestine *Physiol Rev* 1966 *46* 457—512
WANG S C G CLARK, F L. DEY and S W RANSON Further study on the gastro-intestinal motility following stimulation of the hypothalamus *Amer J Physiol* 1940 *130* 81—88

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Colonic Motility in the Cat

II Extrinsic Nervous Control

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Abstract

ROSTAD H *Colonic motility in the cat II Extrinsic nervous control* Acta physiol scand 1973 *89* 91—103

Experiments were performed on cats lightly anesthetized with penthobarbital sodium and a neuromuscular blocking agent pancuronium bromide in order to study the distribution of the extrinsic colonic nerves and their influence on the motility of various parts of the colon The motility was recorded in its proximal middle and distal part with sensitive extraluminal strain gage transducers and arterial blood pressure and respiratory movements were recorded using pressure transducers The influence of the extrinsic nerves on the colonic motility was investigated both by sectioning of the nerves and by electrical stimulation of the peripheral ends of the cut nerves There was no marked functional differences between the proximal and distal portions of the colon. The vagal and splanchnic nerves containing pure motor and inhibitory fibres respectively have their functional distribution throughout the greater part of the colon although most marked in the proximal part The parasympathetic pelvic nerves supply the whole colon with motor fibres At just suprathreshold stimulation of these nerves the responses in the proximal part of the colon were of lower amplitude and longer latency than in the remaining portions The sympathetic lumbar colonic nerves distribute to all parts of the colon with mainly inhibitory fibres but have some excitatory fibres as well

In a study of the central nervous control of the colonic motility in the cat to be reported in subsequent communications it was found necessary to reinvestigate some aspects of the extrinsic nervous control of the motility

The nervous control of the colon in the cat is far more complex than that of the stomach and the small intestine in the sense that the colon has a dual parasympathetic as well as a dual sympathetic innervation (Fig 1)

The main *parasympathetic* supply is conveyed via the pelvic nerves which usually arise from the 2nd and 3rd ventral sacral roots (Langley and Anderson 1895 Schmidt 1933) The fibres reach the more proximal part of the colon by way of the wall of the bowel (Learmonth and Markowitz 1930) The pelvic nerves exert an excitatory control of the entire colon (M Fadden Loughridge and Milroy 1935 Hulten 1969) The motor effects of nerve stimulation are only partly blocked by

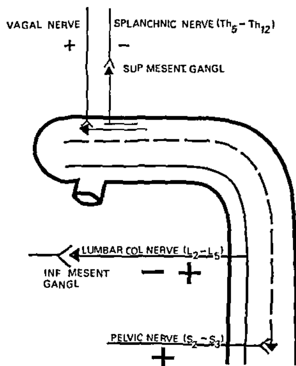


Fig 1 Schematic diagram of the extrinsic innervation of the colon in the cat +motor effects −inhibitory effects

indicating that an unknown noncholinergic transmitter is involved (Hulten 1969 Bennett and Fleshler 1971)

The main *sympathetic* supply arises from the 2nd to the 5th ventral lumbar roots After synaptic interruption in the inferior mesenteric ganglia the postganglionic fibres forming the lumbar colonic nerves accompany the inferior mesenteric artery to the colon (Trumble 1934) These nerves supply the whole colon with inhibitory fibres (Garry 1933 Garry and Gillespie 1955) However after administration of adrenergic blocking agents additional motor effects have been demonstrated by stimulation of the lumbar colonic nerves (Varagic 1956 Day and Rand 1961) Excitation of the colon induced by cerebral stimulation may be conveyed via these sympathetic lumbar colonic nerves (Rostad 1973 b c)

The paired hypogastric nerves arising from the inferior mesenteric ganglia run downwards to join the pelvic plexus They innervate the pelvic organs and the sphincter muscles in the anal canal (Trumble 1934) but do not seem to influence the colonic motility (Garry 1933 Hulten 1969)

Parasympathetic vagal and sympathetic splanchnic fibres (from the 5th to the 12th ventral thoracic roots) reach the colon along the proximal vascular supply (Klee 1913 Schmidt 1933) However controversial results have been reported concerning their influence on the colonic motility Stimulation experiments did not reveal any effects on the colonic motility in dogs and rabbits (Bayliss and Starling 1900 Elliot and Barclay Smith 1904 Wells *et al* 1942) while Hulten (1969) using

cats demonstrated motor and inhibitory responses in the proximal part of the colon with vagal and splanchnic stimulation respectively

In order to determine the peripheral pathways of the colonic responses obtained by central nervous stimulation a knowledge of the effects exerted by the various extrinsic nerve on the various parts of the colon was deemed necessary With sensitive extraluminal strain gage transducers segmental recording of colonic motility has been made possible (Rostad 1973 a)

The differential distribution of the extrinsic nerves to the proximal and distal portions of the colon along with the differences in response of the colon to stimulation of various extrinsic nerves suggest that the organ is comprised of 2 functionally different parts (Hulten 1969) Furthermore differences in the motility response of the proximal and distal human colon to intravenous administration of mecholyl and neostigmine has been reported (Fink and Friedman 1960)

The aim of the present investigation has been to study the influence of selective section and stimulation of the extrinsic colonic nerves on the motility in various segments of the colon in order to clarify the distribution and function of these nerves In addition the effects of some pharmacological agents on colonic response have been tested

Material and methods

The results are based on data gained from 66 cats weighing between 1.8 and 4.9 kg usually more than 3 kg A number of them were also used in the study of central nervous control of motility The preoperative regime was as described in a previous report (Rostad 1973 a) The animals were usually anesthetized with an i.p injection of penthobarbital sodium (Nembutal® Abbott) 30—35 mg/kg b w Sometimes chloralose 30—40 mg/kg b w was given i.v after induction with ether instead of penthobarbital sodium A neuromuscular blocking agent, pancuronium bromide (Pavulon® Organon) was administered i v in amounts of 0.1—0.3 mg/kg b w in order to keep the animal lightly anesthetized and to prevent deep abdominal respiratory movements and skeletal muscle contractions The blocking agent did not influence the effects of section or stimulation of the nerves

The *operative procedures* and *recording technique* were as reported previously (Rostad 1973 a) The arterial blood pressure and respiratory movements were recorded as well as the colonic motility in the proximal the middle and the lower part of the bowel As the pelvic colon is known to contract considerably in the longitudinal direction by pelvic nerve stimulation (Hulten 1969) a transducer was also fixed to the colon sigmoideum in the longitudinal axis in a few animals

The catecholamines from the adrenal glands were excluded from the circulation by careful ligation of all vessels of the glands Adrenocortical substitution was given by intravenous injection of hydrocortisone (Solu-Cortef® Upjohn) in doses of 5—10 mg/kg b w

Other drugs were administered i v atropine (Atropin® 0.1 [illegible] NAF) 0.5—1.0 mg/kg b w guanethidine (Ismelin® Ciba) 4—6 mg/kg b w neostigmine bromide (Neostigmin® 0.05 [illegible] NAF) 0.01—0.2 mg/kg b w

Nerve section and stimulation The *vagal nerves* were dissected free from the sympathetic fibres in the neck, and the *pelvic nerves* were freed as near the sacral roots as possible The preganglionic portion of the *splanchnic nerves* was found beneath the diaphragm and the *lumbar colonic nerves* were carefully dissected free from the inferior mesenteric artery After freepreparation of a nerve a thread was usually placed around it to facilitate its section during the experiment

For bilateral stimulation after division the peripheral ends of the nerves were thread through a short polyethylene tube with 2 circular silver electrodes embedded on its inner wall about 2 mm apart Electrical square wave pulses were employed They were varied with regard to frequency (1—30 Hz) pulse duration (1—10 ms) and intensity (1—20 V) The frequency and pulse duration most commonly used were 10—20 Hz and 2 ms respectively as these parameters were found to be most efficient

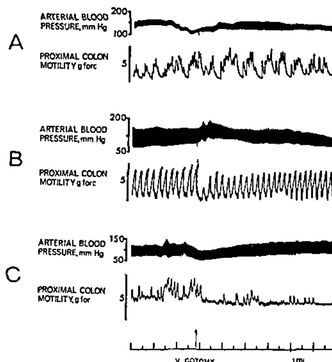


Fig 2 Effects on the proximal colon motility of bilateral cervical vagotomy (A) Cat 3 kg No influence on the motility (B) Cat 4 kg A small decrease of the amplitude (C) Cat 4 1 kg Reduction of the motility

Results

…ction and electrical stimulation of the extrinsic colonic nerves usually did not …gnificantly influence the pulse rate and blood pressure Occasionally slight alterations in blood pressure were seen The colonic effects observed were therefore not secondary to circulatory changes The marked bradycardia and blood pressure fall usually associated with cervical vagal stimulation was almost abolished by pancuronium bromide presumably due to a stabilizing effect on the postsynaptic membrane of the cardiac nerve endings (Bonta Goorissen and Derkx 1968)

The non denervated colon observed with the sensitive extraluminal strain gage technique was usually relatively active and thus provided a good background for *studying the effects of sectioning excitatory nerves and stimulation of inhibitory nerves*

1 Section of extrinsic nerves

Since an increased colonic activity was present immediately after laparotomy (Rostad 1973 a) the nerves in question were usually not cut until 1/2—1 h after the end of the operation

a The parasympathetic nerves — Bilateral cervical *vagotomy* (55 cats) usually did not change the colonic motility However in 7 animals a short lasting and slight reduction of the activity was observed and then usually in the proximal part only

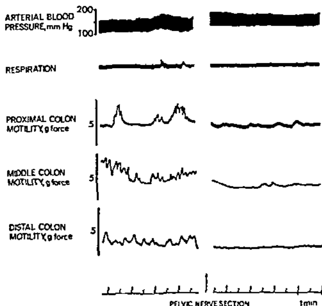


Fig 3 Cat 2 8 kg Effects on the colonic motility of bilateral section of the pelvic nerves Note the reduced activity in *all* segments of the colon

(Fig 2) The result of vagotomy was the same whether or not the other extrinsic nerves had been previously sectioned

Pelvic nerve section during the experiment (18 animals) required a surgical interference which usually resulted in an increased colonic motility of several minutes duration The effect of section of the pelvic nerves *per se* was therefore because of this complication somewhat difficult to evaluate However in 5 experiments some reduction in colonic motility was observed as a result of this procedure (Fig 3) In 2 of these cats a considerable colonic activity was present before the sectioning because the lumbar colonic nerves had been divided immediately beforehand

This suggests that the parasympathetic nerve supply contributes to the spontaneous motility of the colon at least under the experimental conditions present in these experiments

b *The sympathetic nerves* — Section of the *splanchnic nerves* preganglionically (30 cats) usually caused an immediate increase of the colonic activity (21 animals) characterized by sustained contraction waves with rhythmic waves surmounted (Fig 4) This effect was most conspicuous in the proximal part of the colon (10 animals) but was often seen in the lower part of the colon as well (11 animals) To avoid caudad transmission of contractions induced from the proximal segment a ligature was placed at the middle of the bowel The activity gradually subsided again in the course of 15—20 min

Section of the *lumbar colonic nerves* (40 cats) resulted in a considerable increase of activity in the entire colon (35 animals) The changes were in principle the same as those caused by splanchnic nerve section although more pronounced especially

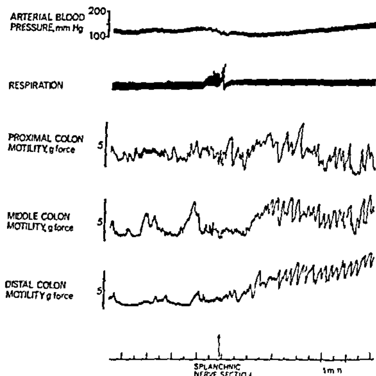


Fig 4 Cat 2 5 kg Augmentation of activity in all parts of the colon after bilateral splanchnic nerve section

in the lower half where the lengthwise shortening movements were more marked In addition the activity lasted 10—20 min longer than by section of the splanchnic nerves In 5 animals section of the lumbar colonic nerves had no significant effect in 2 of these experiments the splanchnic nerves had been cut a few minutes prior to the sectioning The augmented colonic activity caused by the latter procedure was not increased further by additional section of the lumbar colonic nerves

The result of complete sympathectomy was almost the same whether the parasympathetic nerves had been previously divided or not

Section of the paired *hypogastric nerves* (10 animals) never influenced the colonic activity

After *complete extrinsic denervation* of the colon the pattern of motility appeared to be approximately the same as that present before the denervation and which was described in an earlier communication (Rostad 1973 a)

2 Extrinsic nerve stimulation

a The parasympathetic nerves — Stimulation of the *vagal nerves* (37 animals) caused responses that were most conspicuous in the proximal part but in more than half of the experiments also present in the lower part of the colon (Fig 5) After a latency of a few seconds the responses started simultaneously at all recording sites

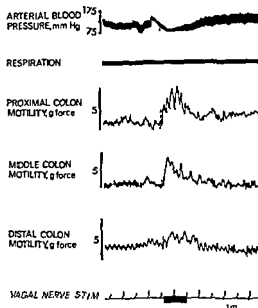


Fig 5 Cat 2 6 kg Effects on the colonic motility of cervical vagal stimulation (10 Hz 2 ms 5 V) Note the response also in the lower part of the colon

and were characterized by slow sustained contractions with rhythmic waves super imposed On cessation of stimulation the response gradually subsided in the course of 30—40 sec The response of the lower portion of the colon was not prevented by a loose ligature in the middle of the bowel Sometimes (7 animals) the response was present in the proximal part only and in a few experiments (7) colonic effect could not be obtained at all by vagal stimulation This lack of effect was usually attributed to deep anesthesia or poor condition of the animal at the end of the experiment Previous section of any or all of the other extrinsic nerves usually did not influence the effect of vagal nerve stimulation and the effect of vagal nerve stimulation was easily blocked by atropine

Uni or bilateral stimulation of the *pelvic nerves* (42 animals) with intensities of more than 4—5 V caused an almost immediate and simultaneous contraction in all parts of the colon Aside from lengthwise contractions in the colon sigmoideum the responses were similar in all segments sustained contractions which slowly died away on cessation of stimulation However at just suprathreshold stimulation (0 5—1 0 V) the responses were more like those caused by vagal stimulation *i e* slow contractions with rhythmic waves superimposed especially in the proximal part Further with such weak stimulation the contractions in the proximal part were of lower amplitude and longer latency (10—20 s) than in the remaining portions (Fig 6) Inhibitory effects were never obtained by pelvic nerve stimulation although the parameters were varied over a wide range

After administration of atropine (17 animals) the response to pelvic nerve stimulation was reduced and delayed but was not completely blocked as was the case with the vagal response However when the animal s condition was poor towards

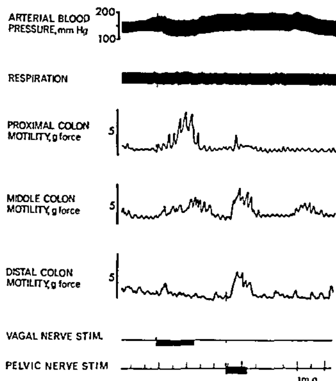


Fig 6 Cat 2 3 kg Effects on the colonic motility of stimulation of the cervical vagal (10 Hz 2 ms 1 5 V) and pelvic nerves (10 Hz, 2 ms 1 V) Note the different effects on the proximal and distal parts of the colon.

the end of the experiment atropine was found in a few experiments to abolish the esponse of pelvic nerve stimulation

Neostigmin in i v doses of 0 01—0 2 mg/kg b w caused a significant increase in the activity in all parts of the colon (25 animals) This was the case whether the colon had been previously denervated or not The increased activity started simultaneously in all parts of the bowel within 1/2—2 min after the injection and slowly subsided in the course of 40—60 min No differences in the response of the proximal and distal parts of the colon were seen irrespective of the amount injected as long as this was effective The colonic response of vagal and pelvic nerve stimulation was potentiated by the drug

b The sympathetic nerves — Bilateral stimulation of the *splanchnic nerves* (5 animals) resulted in inhibition of both the spontaneous colonic motility and the increased activity induced by parasympathetic nerve stimulation The inhibition was most pronounced in the proximal part of the colon where the rhythmic as well as the slow waves were abolished (Fig 7) However sometimes the inhibitory influence also extended to the lower parts of the bowel (Fig 8) The slow sustained contractions were affected more than the faster rhythmic waves

Stimulation of the *lumbar colonic nerves* (14 animals) caused an almost immediate inhibition of motility in all parts of the colon This applied to spontaneous peristalsis as well as to the increased activity induced by parasympathetic nerve

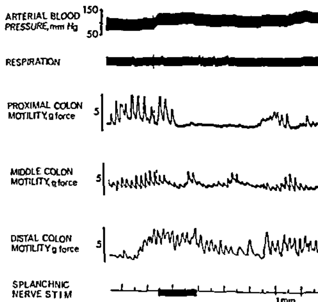


Fig 7 Cat 1 9 kg Inhibition of spontaneous colonic motility by splanchnic nerve stimulation (10 Hz ? ms 15 V) No significant inhibition is seen in the distal part of the colon

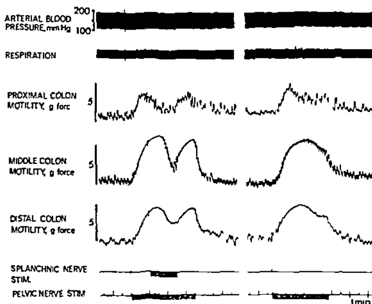


Fig 8 Cat 1 9 kg Effect of splanchnic nerve stimulation on the colonic contractions induced by pelvic nerve stimulation (left) Note inhibition in all parts of the colon Right Result of pelvic nerve stimulation alone Pelvic and splanchnic nerve stimulation (10 Hz 2 ms 15 V)

stimulation This inhibition was completely eliminated by guanethidine (5 cats) In 3 of these experiments lumbar colonic nerve stimulation caused the opposite effect a marked slow contraction of the whole colon

Discussion

The present study using a more sensitive recording technique than previously and allowing simultaneous recording from various segments of the colon has resolved some of the conflicting problems related to the extrinsic innervation of the colon in the cat

(a) The parasympathetic innervation — From the stimulation experiments it seems clear that the entire colon is influenced not only from the sacral division of the parasympathetic system through the pelvic nerves but also from its cranial division through the vagal nerves

The present results of *vagal stimulation* are contrary to the reports of Rentz (1939), Stavney *et al* (1963) and Hulten (1969) who observed excitatory effects of such stimulation in the proximal portion of the colon only in guinea pigs, dogs and cats respectively while Gray *et al* (1955) using dogs sometimes observed a delayed response in the distal colon as well In the present series the responses in the lower part of the colon were not caused by caudad transmission of peristaltic waves. The responses were usually weak and were presumably unveiled mainly because of the sensitive recording technique and the light anesthesia used By deepening the anesthesia the colonic responses to vagal stimulation were abolished or persisted in ⌞ proximal colonic part only

Colonic responses to *pelvic nerve stimulation* were far more readily elicited compared with vagal stimulation When the continuity of the colonic wall was interfered with pelvic nerve stimulation was without effect on the bowel proximal to the site of interruption in accordance with the intramural course of the pelvic nerves A similar arrangement presumably also exists in man (Mitchell 1935 Lannon and Weller 1947) This implies that the proximal colon will be deprived of its pelvic nerve supply on low colonic or rectal resections However it is well known that such surgical procedures usually do not result in functional disturbances of the rest of the colon This is possibly due to the fact that the influence of the pelvic nerves on the proximal part of the colon is less than more distally as indicated by the present threshold stimulation of the pelvic nerves Bayliss and Starling (1900) noted a possible more marked action in the distal than in the upper part of the colon on pelvic nerve stimulation in the cat Similar observations were made by Elliot and Barclay Smith (1904) and Wells *et al* (1942) in the dog while Gray *et al* (1955) observed a delayed effect in the proximal colon on pelvic nerve stimulation

Further, in the present study some difference in the motility response of the proximal and distal part of the colon was obtained on weak pelvic nerve stimulation This observation may support the suggestion of Hulten (1969) that the colon is comprised of 2 functionally different parts

In accordance with previous results (Hulten 1969) atropine abolished the colonic responses of vagal nerve stimulation indicating a cholinergic transmission The effect of pelvic nerve stimulation was to some extent influenced by atropine but was usually not completely eliminated Similar results in dogs were reported by Wells *et al* (1942) Gray *et al* (1955) Goldenberg and Burns (1971)

In isolated colon preparations atropine has been found to abolish the effect of pelvic nerve stimulation (Garry and Gillespie 1955 Lee 1960 Rand and Ridehalgh 1965 Del Tacca *et al* (1968) However atropine resistant excitation has also been demonstrated under comparable experimental conditions (Fulgraff Schmidt and Azokwu 1964 Bennett and Fleshler (1969 b) In addition to cholinergic fibres a noncholinergic excitatory pathway involving an unknown transmitter presumably exists (Bennett and Fleshler 1971) The present study demonstrates that this might be the case also in the intact animal Recent work indicates that prostaglandins may play a role in the gastrointestinal motility (Bennett Eley and Scholes 1968 Bennett Murray and Wyllie 1968 Bennett and Fleshler 1969 a)

An atropine resistant excitatory component in the pelvic nerves probably also exists in the bladder (Edvardsen 1968)

The effect of even small doses of neostigmine bromide on all parts of the colon is in contrast to the observation in man by Fink and Friedman (1960) who reported that small doses of mecholyl and neostigmine selectively enhanced the rhythmic activity of the proximal colon without affecting its distal part They suggested that the concentration of cholinesterase in the distal colon might be greater than in the proximal part However the result may equally well be related to the distribution of the cholinergic vagal nerves The effect of anticholinesterase agents are known to act at the intrinsic ganglion cells and at the muscle fibres as a result of the preservation of acetylcholine released by the cholinergic pre and postganglionic fibres respectively (Koelle 1970) Furthermore according to recent studies in guinea pigs the content of cholinesterase in the gut wall seems to be lower in the distal than in the proximal part of the intestine (Ambache Freeman and Hobbiger 1971) Therefore the effects of neostigmine observed in the present study support the assumption of vagal nerve distribution to the entire colon

Vagotomy has been reported not to have any effect on colonic motility in humans or animals (Grace *et al* 1949 Scott and Cantrell 1949 Gray *et al* 1955 Hulten 1969) In the present series the moderate reduction of motility in some experiments indicates that the vagal nerves probably exert some influence on the spontaneous basal activity of the colon

Significant effects of *pelvic nerve section* were only exceptionally observed and only when considerable colonic motility was present for instance as a result of previous section of the lumbar colonic nerves Similar observations were made by Garry (1933) while Hulten (1969) could not demonstrate any effects of parasympathetic nerve section on the prevailing activity in either part of the colon On the whole the basal activity of the pelvic nerves seems negligible as also maintained by Hulten (1969)

(b) The sympathetic innervation — The considerable increase of colonic motility resulting from section of the sympathetic nerves indicates that these nerves exert a basic tonal influence on the spontaneous colonic motility This is in agreement with previous observations (Learmonth and Markowitz 1930 Garry 1933, Hulten 1969)

However contrary to earlier reports (Bayliss and Starling 1900 Klee 1913 Koennecke 1922 Hulten 1969) the results of section and stimulation of the splanchnic nerves indicate that these nerves also affect the distal colon although not to the same degree as the proximal part

The blocking of the inhibition by guanethidine indicates an adrenergic transmission

The *motor* response obtained by lumbar colonic nerve stimulation after adrenergic blocking is not fully understood (Varagic 1956 Gillespie and MacKenna 1961 Rand and Ridehalgh 1965) According to Kosterlitz (1968) there is strong evidence for an admixture of cholinergic excitatory fibres in the sympathetic outflow to the colon in the rabbit It is likely that this is also the case in the cat However this cannot be the whole explanation since centrally induced excitatory effects to the colon in the cat are conveyed via noncholinergic fibres in the sympathetic lumbar colonic nerves (Rostad 1973 c)

Solu Cortef® was kindly supplied by The Upjohn Company Kalamazoo

References

AMBACHE N M A. FREEMAN and F HOBBIGER Distribution of acetylcholinesterase and butyrylcholinesterase in the myenteric plexus and longitudinal muscle of the guinea pig intestine *Biochem Pharmacol* 1971 20 1123—1132

BAYLISS W M and E H STARLING The movements and the innervation of the large intestine *J Physiol* (Lond) 1900 *26* 107—118

BENNETT A and B FLESHLER, Action of prostaglandin E_1 on the longitudinal muscle of the guinea pig isolated colon *Brit J Pharmacol* 1969 a. *35* 351P—352P

BENNETT A and B FLESHLER, A hyoscine resistant excitatory nerve pathway in guinea pig colon. *J Physiol* (Lond) 1969 b *203* 62P

BENNETT A. and B FLESHLER, A noncholinergic excitatory nerve pathway in guinea pig colon *Amer J dig Dis* 1971 *16* 550—551

BENNETT A. K. G ELEY and G B SCHOLES Effects of prostaglandins E_1 and E_2 on human guinea pig and rat isolated small intestine *Brit J Pharmacol* 1968 *34* 630—638

BENNETT A J C MURRAY and J H WYLLIE Ocurrence of prostaglandin E_2 in the human stomach and a study of its effects in human isolated gastric muscle *Brit J Pharmacol* 1968 *32* 339—349

BONTA, I L. E M GOORISSEN and F H DERKX Pharmacological interaction between pancuronium bromide and anaesthetics *Europ J Pharmacol* 1968 *4* 83—90

DAY M D and M J RAND Effect of guanethidine in revealing cholinergic sympathetic fibres *Brit J Pharmacol* 1961 *17* 245—260

DEL TACCA, M S LECCHINI G M FRIGO A CREMA and G BENZI Antagonism of atropine towards endogenous and exogenous acetylcholine before and after sympathetic system blockade in the isolated distal guinea pig colon *Europ J Pharmacol* 1968 *4* 188—197

EDVARDSEN P Nervous control of urinary bladder in cats *Acta physiol scand* 1968 *72* 234—247

ELLIOT T R. and E BARCLAY SMITH Antiperistalsis and other muscular activities of the colon. *J Physiol* (Lond) 1904 *31* 272—304

FINK S and G FRIEDMAN The differential effect of drugs on the proximal and distal colon *Amer J Med* 1960 *28* 534—540

FULGRAFF G., L. SCHMIDT and PH AZOKWU Über die atropinresistente neuromuskuläre Übertragung am Pelvicus-Colon Präparat der Katz *Arch int Pharmacodyn* 1964 *149* 537—551

GARRY R C The nervous control of the caudal region of the large bowel in the cat *J Physiol* (Lond) 1933 *77* 422—431
GARRY R C and J S GILLESPIE The responses of the musculature of the colon of the rabbit to stimulation in vitro of the parasympathetic and of the sympathetic outflows *J Physiol* (Lond) 1955 *128* 557—576
GILLESPIE J S and B R MACKENNA The inhibitory action of the sympathetic nerves on the smooth muscle of the rabbit gut its reversal by reserpine and restoration by catechol amines and by dopa. *J Physiol* (Lond) 1961 *156* 17—34
GOLDENBERG M M and R H BURNS Atropine resistant spasm of the dog colon induced by intermittent pelvic nerve stimulation *Life Sci* 1971 *10* 591—600
GRACE W J C W HOLMAN S WOLF and H G WOLFF *The effect of vagotomy on the* human colon *Gastroenterology* 1949 *13* 536—546
GRAY G W L C HENDERSHOT R M WHITROCK and M H SEEVERS Influence of the parasympathetic nerves and their relation to the action of atrop ne in the ileum and colon of the dog *Amer J Physiol* 1955 *181* 679—687
HULTEN L Extrinsic nervous control of colonic motility and blood flow *Acta physiol scand* 1969 Suppl 335 116 pp
KLEE PH Der Einfluss der Splanchnicusreizung auf den Ablauf der Verdauungsbewegungen *Pflugers Arch ges Phys ol* 1913 *154* 552—570
KOELLE G B Anticholinesterase agents Pp 442—465 in Goodman L S and A Gilman (eds) *The pharmacological basis of therapeutics 4th ed* London Toronto The MacMillan Co 1970 1794 pp
KOENNECKE W Experimentelle Innervationsstorungen am Magen und Darm *Z ges exp Med* 1922 *28* 384—412
KOSTERLITZ H W Intrinsic and extrinsic nervous control of motility of the stomach and the intestines Pp 2147—2171 in *Handbook of Physiology* Sect 6 Vol 4 Baltimore Williams and Wilkins Co 1968 2343 pp
LANGLEY J N and H K ANDERSON On the innervation of the pelvic and adjoining viscera *J Physiol* (Lond) 1895 *18* 67—105
LANNON J and E WELLER The parasympathetic supply of the distal colon *Brit J Surg* 1947 *34* 373—378
LEARMONTH J R and J MARKOWITZ Studies on the innervation of the large bowel *Amer J Physiol* 1930 *94* 501—504
LEE C Y The effect of stimulation of extrinsic nerves on peristalsis and on the release of 5 hydroxytryptamine in the large intestine of the guinea pig and of the rabbit *J Physiol* (Lond) 1960 *152* 405—418
MFADDEN G D F J S LOUGHRIDGE and T H MILROY The nerve control of the distal colon *Quart J exp Physiol* 1935 *25* 315—327
MITCHELL G A G The innervation of the distal co on *Edinb med J* 1935 *42* 11—20
RAND M J and A RIDEHALGH Actions of hemicholinium and trieth lcholine on respon es of guinea pig colon to stimulations of autonomic nerves *J Pharm Pharmacol* 1965 *17* 144—156
RENTZ E Über den Einfluss der Vagusreizung auf die Bewegungen des Dickdarms *Naunyn Schmiedebergs Arch exp Path Pharmak* 1939 *191* 172—191
ROSTAD H Colonic motility in the cat I Extraluminal strain gage technique Influence of anesthesia and temperature *Acta physiol scand* 1973 a *89* 79—90
ROSTAD H Colonic motility in the cat IV Peripheral pathways mediating the effects induced by hypothalam c and mesencephalic stimulation *Acta physiol scand* 1973 b *89* 154—168
ROSTAD H Colonic motility in the cat V Influence of telencephal c stimulation and peripheral pathways mediat ng the effects *Acta physiol sca d* 1973 c *89* 169—181
SCHMIDT CH A Distribution of vagus and sacral nerves to th large intestine *Proc Soc exp Biol* (NY) 1933 *30* 739—740
SCOTT H W and J R CANTRELL Colonmetrographic studies of the effects of section of the parasympathetic nerves of the colon *Bull Johns Hopk Hosp* 1949 *85* 310—319
STAVNEY L S T KATO CH A GRIFFITH L M NYHUS and H N HARKINS A physiological study of motility changes following select e gastric agotom *J surg Res* 1963 *3* 390—394
TRUMBLE H C The plan of the visceral nerve n the lumbar and sacral outflo s of the autonomic nervous system *Brit J Su g* 1934 *21* 664—6 6
VARAGIĆ V The effect of tolazoline and other u tances on the response of the isolated colon of the rabb t to n rve stimulation *A ch i t Pl a macodyn* 1956 *106* 141—1 0
WELLS J A T H MERCER J S GRAY and A C IVY The motor innervation of the colon *Amer J Phys ol* 1942 *138* 83—93

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Colonic Motility in the Cat

III Influence of Hypothalamic and Mesencephalic Stimulation

By

HANS ROSTAD

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Abstract

ROSTAD H *Colonic motility in the cat III Influence of hypothalamic and mesencephalic stimulation* Acta physiol scand 1973 *89* 104—115

Colonic motility recorded with sensitive extraluminal strain gage transducers was studied in lightly anesthetized cats following electrical stimulation of hypothalamic and mesencephalic structures Stimulation of various structures of the *hypothalamus* produced the following 1) The sympatho inhibitory area in the anterior hypothalamus increased colonic activity concomitantly with a fall in blood pressure 2) The ventral anterior and lateral hypothalamus produced excitatory colonic effects but these were usually associated with a rise in blood pressure Similar effects were obtained from the dorsal part of the ventromedial hypothalamic nucleus 3) Stimulation of the ventral part of the hypothalamus mainly corresponding to the course of the medial forebrain bundle and the ventral part of the ventromedial nucleus resulted in inhibitory colonic effects Stimulation of various structures of the *mesencephalon* produced the following 1) The central gray substance red nucleus and reticular substance elicited increased colonic effects most conspicuous in the proximal part of the colon These responses were usually associated with a blood pressure rise 2) Points within the central tegmental tract and the medial lemniscus resulted in inhibition of colonic motility The functional significance of the results and their relation to behavioural patterns are discussed

Although the influence of brain stimulation on gastric motility has been the subject of rather extensive studies (for references see Fennegan and Puiggari 1966 Thomas and Baldwin 1968) few reports deal with the cerebral control of colonic motility The areas most commonly found to be involved in the control of gastric motility as well as other autonomic activities are (apart from the lower brain stem) the hypothalamus the mesencephalon the cerebral cortex the amygdaloid nuclear complex and the septal nuclei The present communication is concerned with the hypothalamus and the mesencephalon whereas the telencephalic areas will be treated in a subsequent report (Rostad 1973 d)

Studies of the influence of *hypothalamic* stimulation on the lower part of the gut have not been in complete agreement presumably because of inadequate recording techniques Using ether and chloralose anesthesia and recording with intraluminal balloons electrical stimulation of extensive areas within the hypothalamus in cats

has produced a delayed augmentation of the colonic motility often preceded by an initial inhibition (Wang *et al* 1940 Ingersoll and Jones 1946 Strom and Uvnäs 1950) In unanesthetized cats and using a similar recording technique Sheehan (1942) observed inhibition only of colonic contractions from stimulation of large areas of the hypothalamus On the other hand Boom *et al* (1965) using open ended tubes in unanesthetized cats found only a few excitatory points in the anterior hypothalamus In dogs anesthetized with chloralose excitatory colonic effects were obtained from extensive areas of the hypothalamus (Fang and Kuo 1967)

After *mesencephalic* transection studies in cats Langworthy and Rosenberg (1939) concluded that there was a mechanism in the midbrain controlling tone in the smooth muscle of the rectum With respect to gastric motility stimulation of the superior colliculus and various areas of the ventral part of the mesencephalon in anesthetized cats produced excitatory effects while inhibitory responses were obtained from the central and dorsal parts of the tegmentum the medial lemniscus and the area around the longitudinal medial fascicle (Eliasson 1954)

The *purpose* of the present work was to study the influence of the hypothalamus and mesencephalon on the colonic motility in cats Various parts of the hypothalamus and mesencephalon have been electrically stimulated and the changes in the colonic motility have been recorded utilizing the sensitive extraluminal strain gage transducers previously described (Rostad 1973 a)

Material and methods

The results are based on data gained from 59 cats mostly male weighing between 1.8 and 5.3 kg Some of the animals were also used in the study of the peripheral pathways conveying the hypothalamic and mesencephalic effects (Rostad 1973 c) The preoperative regime was as described in a previous communication (Rostad 1973 a) Most animals were anesthetized with penthobarbital sodium (Nembutal® Abbott) 30 mg/kg b w i p and some with chloralose 30—40 mg/kg b w i v after induction with ether In a few experiments a combination of barbiturate and chloralose anesthesia was used The neuromuscular blocking agent pancuronium bromide (Pavulon® Organon) was administered i v in amounts of 0.1—0.3 mg/kg b w to prevent striated muscle artifacts and thus permitting the use of lightly anesthetized animals Stimulus currents strong enough to influence gastrointestinal motility are known to cause respiratory changes (Mason and Nelsen 1969) which invariably affect the recording in a closed abdominal cavity almost irrespective of the technique utilized Deep anesthesia has to be avoided as it reduces the excitability of the central nervous structures and decreases the spontaneous colonic peristalsis (Rostad 1973 a) making it difficult to observe inhibitory responses

The *operative procedures* and *recording techniques* were as described previously (Rostad 1973 a) In brief the colonic motility in the proximal the middle and the lower part of the bowel was recorded as well as the arterial blood pressure and the respiratory movements The catecholamines from the adrenal glands were excluded from the circulation by careful ligation of the vessels of both glands Adrenocortical substitution was achieved by i v injection of hydrocortison (Solu Cortef® Upjohn) 5—10 mg/kg b w

Electrical stimulation With the animal in the supine position the head and the upper part of the body were gently turned and the head immobilized in a Horsley Clarke frame Craniotomy was performed the dura removed and the cerebral cortex exposed to the extent required For stimulation a monopolar or occasionally a bipolar stainless steel electrode was stereotaxically inserted using the coordinates given by Jasper and Ajmone Marsan (1954) The monopolar electrodes were insulated except for the very tip (40—60 μm) the indifferent electrode being connected to the cut skin of the head The bipolar electrode consisted of a thin cannula with a central core of stainless steel wire (150 μm) both insulated except for the tip The distance between the electrodes measured 1 mm the central core being connected to the cathode

significant differences were observed between the monopolar and bipolar stimulating techniques concerning the size of the responses produced or in the extent of the responsive hypothalamic area.

Square wave pulses at frequencies from 10 to 200 Hz and of 1—10 ms duration were employed and the optimal values both for colonic excitation and inhibition found to be about 80—100 Hz and 1 ms duration. The intensity varied from 1 to 6 V corresponding to current strengths of about 0.05—0.30 mA.

Histology. To identify the points stimulated an anodal current of 1 mA was passed through the electrode for 30 s at the end of the experiment. The brain was then perfused with saline and then with 10 % formalin. The electrode placements were identified in thionine stained sections 20 μm thick, every 20th section being stained. Intervening sections were studied when necessary.

Results

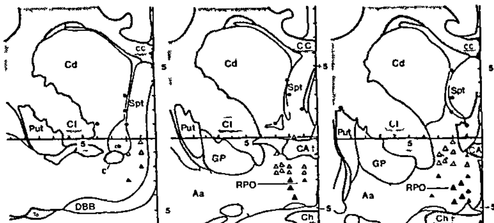
Intensities of 2—3 V were usually sufficient to elicit motor as well as inhibitory colonic responses. The effects could be changed by moving the electrode tip only 1 mm which may serve as a good control of the spread of the stimulating current (Eliasson 1954). An excitatory response could never be reversed into an inhibitory one or *vice versa* by varying the stimulus parameters. The latent period from the beginning of the stimulation to the start of the colonic response was usually less than 5—6 s indicating a nervous pathway (Youmans 1949 p. 120). Various effects on the blood pressure and pulse rate were often seen concomitantly. However these cardiovascular effects started simultaneously with or after the colonic response, thus the latter effects were not secondary to the circulatory ones. Furthermore colonic responses could often be obtained with intensities too weak to elicit any cardiovascular effects.

The hypothalamus

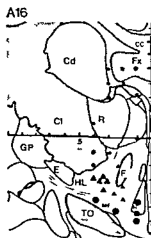
The hypothalamus was explored in 47 cats with about 2 300 stimulations being carried out. Excitatory as well as inhibitory responses were produced. Representative points are illustrated in Fig. 1.

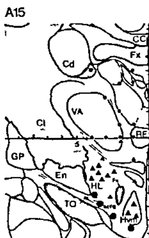
For the *excitatory* effects the following topographical distribution of responsive loci was found. In the *anterior hypothalamus* corresponding to the sympatho-inhibitory area described by Folkow, Johansson and Öberg (1959) with respect to the cardiovascular responses (Fig. 1 A16—A14), augmentatory colonic responses and a concomitant blood pressure fall were regularly observed (Fig. 2 A). Within 5—6 s a conspicuous slow contraction with faster rhythmic waves surmounted started almost simultaneously in all parts of colon. On cessation of stimulation the response disappeared in the course of 30—60 s.

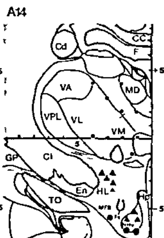
A ventral shift of the electrode tip to the ventral anterior hypothalamus changed the cardiovascular response to one of increased pulse rate and rise in blood pressure whereas the colonic responses were still excitatory but with a lower threshold of excitability than the responses resulting from stimulation of more dorsally located structure (Fig. 2 B). The anterior hypothalamus was stimulated in 44 animals and in 41 of these reproducible excitatory colonic responses were obtained.



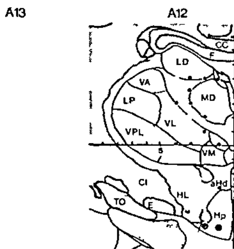
A16 A15 A14

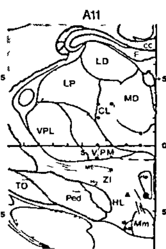






A13 A12 A11





A10

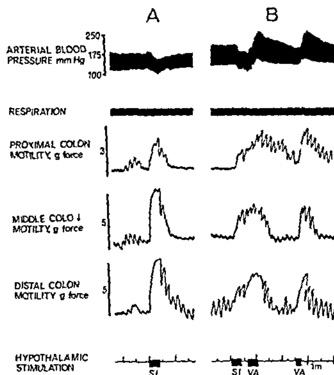


Fig 2 Cat 4 3 kg *A* Augmentatory colonic responses associated with a drop in blood pressure on stimulation of the sympatho-inhibitory area in the anterior hypothalamus (SI) *B* Stepwise lowering of the electrode tip in plane AH from the sympatho-inhibitory zone to the ventral anterior hypothalamus (VA) cf Fig 1 Note the change from an initial fall in blood pressure (SI) to a rise (VA) whereas the colonic responses are all excitatory Stimulus parameters 100 Hz 1 ms 3 V

In the *middle part of the hypothalamus* (17 animals) stimulation of its lateral zone produced excitatory colonic effects the most sensitive points being in the frontal plane A13—A11 (Fig 1) A concomitant blood pressure rise usually appeared after the initiation of the colonic contractions

The dorsal portion of the ventromedial hypothalamic nucleus (A12—A11) proved very sensitive and even weak stimuli (0 3—0 5 V) produced marked colonic contractions In one animal defecation occurred as a result of stimulation of this area The associated blood pressure effects were usually of the pressor type but they were ordinarily weaker compared with the lateral and anterior responsive areas

Stimulation at the level of the *posterior hypothalamus* (A10—A9) (33 animals) with intensities of 3—5 V produced weak excitatory colonic responses from scattered points within its lateral and medial portions The effects on the blood pressure were relatively small and usually augmentatory

Inhibitory colonic responses characterized by relaxation and arrest of rhythmic activity were usually obtained only when abundant colonic background motility wa

Fig 1 Frontal sections *through* the diencephalon of the cat and eating representative points which on electrical stimulation influenced the colonic motility Open triangles Augmentation of colonic motility and a concomitant fall in blood pressure Solid triangles Excitatory colonic responses usually associated with a rise in blood pressure Large triangles indicate the most sensitive points Filled circles Inhibitory colonic effects Dots No response For abbreviations see end of article

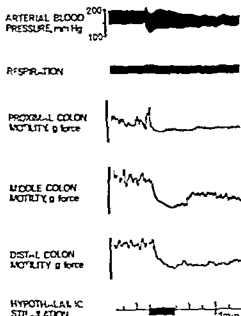


Fig 3 Cat 2.2 kg Inhibitory colonic responses on stimulation in the ventral part of the middle hypothalamus (A13) (30 Hz, 1 ms 3 V)

present (Fig 3) Inhibitory points were traced in an uninterrupted ventral longitudinal zone extending through the middle part of the hypothalamus to its caudal end (A13—A9) The effective points appear to correspond mainly to the course of the medial forebrain bundle Some points were located in the ventral part of the ventromedial hypothalamic nucleus The pattern of blood pressure usually observed concomitant to stimulation was an initial fall followed by a moderate rise

B The mesencephalon

Mesencephalic structures were explored in 18 animals with about 900 stimulations Representative points are illustrated in Fig 4

Excitatory responses most conspicuous in the proximal part of the colon were obtained from various areas of the mesencephalon The lowest threshold points were located in the central gray substance (Fig 5 A but colonic excitation was also induced by stimulation within the reticular substance and magnocellular part of the red nucleus The motility responses were usually associated with a rise in blood pressure but sometimes a moderate hypotensive effect was observed as exemplified in Fig 5

Inhibitory effects on the activity in all parts of the colon were usually encountered by stimulation of points which seemed to be localized mainly within the central tegmental tract and the medial lemniscus Fig 5 B A concomitant rise in blood pressure and increased pulse rate was observed following stimulation in this area

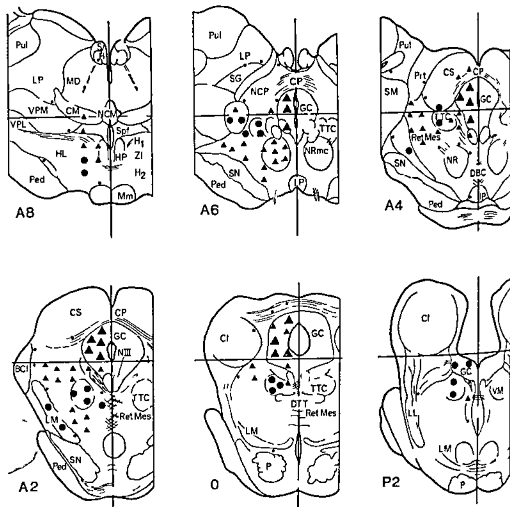


Fig 4 Frontal sections through the mesencephalon of the cat showing the distribution of representative points yielding excitatory and inhibitory colonic effects Outline of drawings from Kaada *et al* (1967) Solid triangles Excitatory colonic responses usually associated with a rise in blood pressure Large triangles indicate the most sensitive points Filled circles Inhibitory colonic effects Dots No response For abbreviations see end of article

Discussion

The present findings indicate a more restrictive localization of the hypothalamic areas influencing colonic motility than described in previous reports (see introduction) This is probably due to the sensitive recording technique thus permitting the use of weaker stimulus strengths Further a lighter anesthesia could be used since artifacts due to skeletal muscle contractions were prevented by a neuromuscular blocking agent

A The hypothalamus

The excitatory colonic responses obtained from the sympatho inhibitory area within

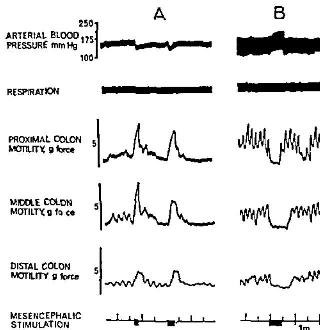


Fig 5 *A* Cat 2 9 kg Excitatory colonic responses resulting from stimulation within the central gray substance of the mesencephalon (100 Hz 1 ms 3 V) The contractions are most marked in the proximal half of the colon *B* Cat 2 6 kg Inhibitory colonic responses on stimulation within the central tegmental tract of the mesencephalon (80 Hz 1 ms 3 V)

the anterior hypothalamus might be caused by a reduction of sympathetic tone since this zone seems to exert a disinhibitory effect by suppressing an adrenergic background discharge (Jansson Lisander and Martinson 1969) The blood pressure fall in the present series was less marked than that described by Folkow Johansson and Öberg (1959) possibly because of the stabilizing effect of pancuronium bromide (Rostad 1973 b) However a moderate fall in blood pressure was also observed in cats anesthetized with chloralose (Hilton and Spyer 1971)

The more ventrally located area from which augmentatory colonic responses associated with increased blood pressure were elicited seems to correspond to the defense area as first outlined by Hess and Brugger (1943) Abrahams Hilton and Zbrozyna (1960) included the behaviour patterns of flight as well as of attack in the term defense reaction Stimulation of this apparently same anterior hypothalamic area has previously been shown to evoke an excitatory influence on the uterine motility in rabbits (Setekleiv 1964) and the gastric motility in dogs (Fennegan and Puiggari 1966) On the other hand Jansson Lisander and Martinson (1969) obtained inhibition of vagally induced gastric activity in cats when stimulating the apparently same anterior hypothalamic zone Stimulation of this defense area is known to produce circulatory changes including rises in arterial blood pressure and a reduction of mesenteric blood flow (Cobbold *et al* 1964) However the increased colonic motility recorded in the present series of experiments does not appear to be secondary to circulatory changes as the effects could be obtained with lower stimulus intensities and had a shorter latency than the cardiovascular responses

The lateral hypothalamic area from which marked excitatory colonic effects were

induced appears to coincide with the so-called feeding center (Brügger 1943 Anand and Brobeck 1951 Grossman 1962) Stimulation of this area elicits eatin and a marked increase in the motility of the stomach and small intestine (Folkow and Rubinstein 1965) After damage to the same zone the animals refuse to eat and die even in the presence of food and water (Anand Dua and Schoenberg 1955) Thus with respect to the responsive lateral hypothalamic area there seems to be a correspondance between food intake and the motility of the gastrointestinal tract including the colon

With regard to the ventromedial hypothalamic nucleus electrical stimulation in unanesthetized animals causes a decrease in food intake (Anand and Dua 1955) while bilateral lesions produce hyperphagia and obesity primarily caused by over eating (for references see Grossman 1967 pp 311—394) Stimulation of the dorsal part of the ventromedial hypothalamic nucleus elicited mainly excitatory colonic responses in the present experiments as well as in those of Wang *et al* (1940) and Fang and Kuo (1967) using anesthetized cats and dogs respectively Ventromedial hypothalamic stimulation was similarly found to increase gastric motility (Fennegan and Puiggari 1966)

Hypothalamic points responsible for inhibitory colonic effects were traced in the ventral part of the hypothalamus mainly corresponding to the course of the medial forebrain bundle and the ventral part of the ventromedial nucleus The medial fore brain bundle is the pathway for a massive projection of direct cortico hypothalamic fibres from the posterior orbital and premotor frontal cortex to the ventromedial -pothalamic nucleus (Le Gros Clark and Meyer 1950) As stimulation of both these cortical areas has been found to produce colonic inhibition (Rostad 1973d) the effects obtained from the medial forebrain bundle might be due to activation of this cortico-hypothalamic fibre system.

In a study of behavioural and autonomic responses in the cat Folkow and Rubin tein (1965) observed defense reactions and inhibition of gastric and small in testinal motility on stimulation around the perifornical region and the medial fore brain bundle in the ventral part of the lateral hypothalamus However other in vestigators distinguish two types of agonistic behaviour elicitable from separate points within this area a defense (rage) reaction and a flight (fear) reaction (Romaniuk 1963 1965) Defense reponses are obtained by stimulation of the ventral part of the hypothalamus and the flight re ponses from more dorsal parts (Romaniuk 1965) These areas seem to correspond to those responsible for colonic inhibition and ex citation respectively Colonic inhibition therefore seems to be associated with the reaction of defense while flight and fear seem to be accompanied by an increased colonic motility It is of interest to recall that defecation as well as urination mi ht occur in a condition of intense fear (Zbrozyna 1972)

Lesions in various portions of the hypothalamus are known to produce gastro intestinal erosions and submucosal hemorrhages (Sheehan 1940 French *et al* 195? Feldman Behar and Birnbaum 1961) However these effects are presumably due to secretory disturbances (Gray *et al.* 1951) and not to motility changes

B The mesencephalon

The motility responses from mesencephalic structures were usually weaker than those obtained by hypothalamic stimulation The central gray substance responsible for the most marked excitatory colonic responses receives afferent fibres from other areas exerting a facilitatory influence on the motility of colon *i.e.* the amygdala (Rostad 1973 d) and the lateral hypothalamic region (Nauta 1958) The central gray substance also sends fibres to the lateral hypothalamus

The central gray substance has also been found to produce motor effects on bladder motility in cats (Gjone 1966) whereas the gastric motility seems almost uninfluenced (Eliasson 1954)

However with regard to the inhibitory effects the gastric motility appears to be affected by the same mesencephalic structures as the colonic motility *i.e.* the central tegmental tract and the medial lemniscus (Eliasson 1954)

The inhibitory colonic effects of mesencephalic stimulation are in accordance with the assumption of an inhibitory tonic mechanism in the midbrain controlling the smooth muscle of the rectal wall (Langworthy and Rosenberg 1939) In their study of the rectal motility in cats transection of the brain stem through the mesencephalon caused a hyperactivity which was subsequently abolished by transection below the midbrain

Finally in the present experiments the rise in blood pressure observed concomitantly with the colonic inhibitory effects of mesencephalic stimulation indicates a sympathetic activation which tends to suppress gastrointestinal motility

The peripheral pathways mediating the colonic effects obtained by hypothalamic and mesencephalic stimulation are dealt with in a subsequent report (Rostad 1973 c)

Solu Cortef® was kindly supplied by The Upjohn Company Kalamazoo

References

ABRAHAMS V C S M HILTON and A ZBROZYNA Active muscle vasodilatation produced by stimulation of the brain stem its significance in the defence reaction *J Physiol* (Lond) 1960 *154* 491—513

ANAND B K and J R BROBECK Hypothalamic control of food intake in rats and cats *Yale J Biol Med* 1951 *24* 123—140

ANAND B K and S DUA Feeding responses induced by electrical stimulation of the hypothalamus in the cat *Indian J med Res* 1955 *43* 113—122

ANAND B K S DUA and K SCHOENBERG Hypothalamic control of food intake in cats and monkeys *J Physiol* (Lond) 1955 *127* 143—152

BOOM R G CHAVEZ IBARRA J J DEL VILLAR and R HERNANDEZ PEON Changes of colonic motility induced by electrical and chemical stimulation of the forebrain and hypothalamus in cats *Int J Neuropharmacol* 1965 *4* 169—175

BRUGGER M Fresstrieb als hypothalamisches Symptom *Helv physiol pharmacol Acta* 1943 *1* 183—198

CLARK W E LE GROS and M MEYER, Anatomical relationships between the cerebral cortex and the hypothalamus *Brit med Bull* 1950 *6* 341—345

COBBOLD A B FOLKOW O LUNDGREN and I WALLENTIN Blood flow capillary filtration coefficients and regional blood volume responses in the intestine of the cat during stimulation of the hypothalamic defense area *Acta physiol scand* 1964 *61* 467—475

ELIASSON S Activation of gastric motility from the brain stem of the cat *Acta physiol scand* 1954 *30* 199—214

FANG H S and Y F KUO Colonic motility following hypothalamic stimulation in the dog *Aerospace Med* 1967 *38* 812—814
FELDMAN S A J BEHAR and D BIRNBAUM Gastric lesions following hypothalamic stimulation *Arch Neurol* (Chic) 1961 *4* 308—317
FENNEGAN F M and M J PUIGDARI Hypothalamic and amygdaloid influence on gastric motility in dogs *J Neurosurg* 1966 *24* 497—504
FOLKOW B B JOHANSSON and B ÖBERG A hypothalamic structure with a marked inhibitory effect on tonic sympathetic activity *Acta physiol scand* 1959 *47* 262—270
FOLKOW B and E H RUBINSTEIN Behavioural and autonomic patterns evoked by stimulation of the lateral hypothalamic area in the cat *Acta physiol scand* 1965 *65* 292—299
FRENCH J D R W PORTER F K VON AMERONGEN and R B RANEY Gastrointestinal hemorrhage and ulceration associated with intracranial lesions a clinical and experimental study *Surgery* 1952 *32* 395—407
GJONE R Excitatory and inhibitory bladder responses to stimulation of limbic diencephalic and mesencephalic structures in the cat *Acta physiol scand* 1966 *66* 91—102
GRAY S J J A BENSON JR R W REIFENSTEIN and H M SPIRO Chronic stress and peptic ulcer I Effect of corticotropin (ACTH) and cortisone on gastric secretion *J Amer med Ass* 1951 *147* 1529—1537
GROSSMAN S P Direct adrenergic and cholinergic stimulation of hypothalamic mechanisms *Amer J Physiol* 1962 *202* 872—882
GROSSMAN S P *A Textbook of physiological Psychology* New York London Sydney John Wiley & Sons Inc 1967 932 pp
HESS W R and M BRUGGER Das subkortikale Zentrum der affektiven Abwehrreaktion *Helv physiol pharmacol Acta* 1943 *1* 33—52
HILTON S M and K M SPYER Participation of the anterior hypothalamus in the baroreceptor reflex *J Physiol* (Lond) 1971 *218* 271—293
INGERSOLL E H and L JONES The effect upon the colon of electrical stimulation of fore brain areas in the cat *Amer J Physiol* 1946 *146* 187—191
JANSSON G B LISANDER and J MARTINSON Hypothalamic control of adrenergic outflow to the stomach in the cat *Acta physiol scand* 1969 *75* 176—186
JASPER H H and C AJMONE MARSAN *A stereotaxic atlas of the diencephalon of the cat* Ottawa National Research Council of Canada 1954
KAADA B R F THOMAS E ALNÆS and K WESTER EEG synchronization induced by high frequency midbrain reticular stimulation in anesthetized cats *Electroenceph clin Neuro physiol* 1967 *22* 220—230
LANGWORTHY O R and S J ROSENBERG Control by the central nervous system of rectal smooth muscle *J Neurophysiol* 1939 *2* 356—360
MASON G R and T S NELSEN Gastric secretory and motor responses to anterior hypothalamic stimulation *Amer J Physiol* 1969 *217* 1771—1775
NAUTA W J H Hippocampal projections and related neural pathways to the mid brain in the cat *Brain* 1958 *81* 319—340
ROMANIUK A Emotional responses evoked by hypothalamic stimulation in cats *Bull Acad pol Sci* 1963 *11* 437—440
ROMANIUK A Representation of aggression and flight reactions in the hypothalamus of the cat *Acta Biol exp* (Warszawa) 1965 *25* 177—186
ROSTAD H Colonic motility in the cat I Extraluminal strain gage technique Influence of anesthesia and temperature *Acta physiol scand* 1973 a *89* 79—90
ROSTAD H Colonic motility in the cat II Extrinsic nervous control *Acta physiol scand* 1973 b *89* 91—103
ROSTAD H Colonic motility in the cat IV Peripheral pathways mediating the effects induced by hypothalamic and mesencephalic stimulation *Acta physiol scand* 1973 c *89* 154—168
ROSTAD H Colonic motility in the cat V Influence of telencephalic stimulation and peripheral pathways mediating the effects *Acta physiol scand* 1973 d *89* 169—181
SETEKLEIV J Uterine motility in the estrogenized rabbit V Response to brain stimulation *Acta physiol scand* 1964 *62* 313—322
SHEEHAN D The hypothalamus and gastro-intestinal regulation *Res Publ Ass nerv ment Dis* 1940 *20* 589—616
SHEEHAN D Relationship of the hypothalamus to the large bowel *Amer J dig Dis* 1942 *9* 361—363
STROM G and B UVNÄS Motor responses of gastro-intestinal tract and bladder to topical stimulation of the frontal lobe basal ganglia and hypothalamus in the cat *Acta physiol scand* 1950 *21* 90—104
THOMAS J E and M V BALDWIN Pathways and mechanisms of regulation of gastric motility

Pp 193,—1968 in *Handbook of Physiology* Sect 6 Vol 4 Baltimore Williams and Wilkins Co 1968 2343 pp

WANG S C G CLARK F L DEY and S W RANSON Further study on the gastro-intestinal motility following stimulation of the hypothalamus *Amer J Physiol* 1940 *130* 81—88

YOUMANS W B *Nervous and neurohormonal regulation of intestinal motility* New York London Interscience Publishers Ltd 1949 129 pp

ZBROZYNA A W The organization of the defence reaction elicited from amygdala and its connections Pp 597—606 in Eleftheriou B E (ed) *The neurobiology of the amygdala* New York London Plenum Press 1972 819 pp

Abbreviations for Fig 1 and 4 (Nomenclature according to Jasper and Ajmone Marsan 1954)

Aa area amygdaloidea anterior
Acb n accumbens
aHd area hypothalamica dorsalis
AL ansa lenticularis
BCI brachium colliculi inferioris
CA commissura anterior
CC corpus callosum
Cd n caudatus
Ch chiasma opticum
CI (Fig 1) capsula interna
CI (Fig 4) colliculus inferior
CL n centralis lateralis
CM n centrum medianum
CP commissura posterior
CS colliculus superior
DBB diagonal band of Broca
DCB decussatio brachiorum conjunctivorum
DTT decussatio tractus tegmentalis
En n entopeduncularis
Fx fornix
GC griseum centrale
GP globus pallidus
H_1 H Forel s fi lds
Ha hypothalamus anterior
HL hypothalamus lateralis
Hp hypothalamus posterior
Hvm hypothalamus ventromedialis
IP b interpeduncularis
LM lemniscus medialis
LME lamina medullaris externa
LP n lateralis posterior
MD n medialis dorsalis
MFB medial forebrain bundle
Ml n mamillaris lateralis
Mm corpus mamillare
N III n third cranial nerve
NHvm n hypothalami ventromedialis
NRmc n ruber
P n posterior
Ped pedunculus cerebralis
Prt praetectum
Pul pulvinar
Put putamen
R n reticularis
RE n reuniens
Ret.Mes substantia reticularis mesencephalica
RPO regio preoptica
SG n suprageniculatus
SM n submedius
SN substantia nigra
Spt area septalis
TO tractus opticus
Tof tractus olfactorius
TTC tractus tegmentalis centralis
VA n ventralis anterior
VL n ventralis lateralis
VM n ventralis medialis
VPL n ventralis postero-lateralis
VPM n ventralis postero-medialis
ZI zona incerta

Acta physiol scand 1973 89 116—125

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Driving Forces over the Peritubular Capillary Membrane in the Rat Kidney during Antidiuresis and Saline Expansion

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Abstract

KÄLLSKOG Ö and M WOLGAST *Driving forces over the peritubular capillary membrane in the rat kidney during antidiuresis and saline expansion* Acta physiol scand 1973 *89* 116—125

The driving forces operative over the renal peritubular capillary membrane has been investigated on a series of male Sprague Dawley rats The hydrostatic capillary pressure was measured by micropuncture technique using a servo-controlled counter pressure device according to Wiederhielm The oncotic pressure was estimated from the protein concentration calculated as the arithmetic mean of the concentration in the proximal end (= plasma concentration /1— filtration fraction) and that in the distal end of the capillary (= plasma concentration

For estimation of the corresponding interstitial variables a small (50 μm) PCV catheter was put into the subcapsular space The hydrostatic pressure was measured with the same system as for capillaries and the oncotic pressure from the protein concentration in samples withdrawn via the microcatheter Under control antidiuretic conditions the capillary hydrostatic pressure averaged 11 1 mm Hg and the oncotic 27 7 mm Hg In the interstitium the corresponding pressures amounted 1 3 mm Hg and 4 0 mm Hg respectively In saline expansion the interstitial hydrostatic pressure almost doubled whereas the oncotic pressure decreased towards zero partly compensating the decrease in net driving force caused by the decreased plasma oncotic pressure following the expansion Data from lymph flows suggest that the capillary membrane has a small and a large pore system and from these suggestions a model for the regulation of renal interstitium is presented

During recent years considerable evidence has accumulated supporting the idea of a peritubular control of preferentially proximal fluid reabsorption in the sense that the removal of tubular reabsorbate from the renal interstitium to a large extent determines the fractional and absolute proximal fluid reabsorption This removal is in turn determined by the driving forces for fluid reabsorption operating over the peritubular capillary membrane (*e g* Lewy and Windhager 1968 Spitzer and Windhager 1970 Bank *et al* 1969 Daugharty *et al* 1968 Falchuk *et al* 1971 Brenner *et al* 1971 Daugarthy *et al* 1972)

Briefly stated manoeuvres which increase the net driving force over the capillary membrane also enhance proximal fluid reabsorption and vice versa This hypothesis explains several important features in the control of proximal reabsorption Spon

taneous increase for instance in the glomerular filtration rate leads to increa ed ab solute proximal reabsorption as well According to the hypothe is this increased re absorption is due to the increased postglomerular protein concentration and thereby increased intravascular mean oncotic pressure (Lewy and Windhager 1968) Extra cellular volume expansion with saline or equivalent solutions causing a decreased peritubular colloid osmotic pressure will acordingly be concomitant with a decreased proximal reabsorption A decreased peritubular capillary hydrostatic pressure will in the same way lead to an enhanced proximal tubular reabsorption

This phenomenon is restricted to the control of sodium and fluid reabsorption in proximal tubules The distal tubules seem to be unaffected or rather respond in the opposite manner with an increased sodium reabsorption with increasing distal load as cau ed by the depressed proximal reabsorption (Brenner and Berliner 1969)

It ought to be emph isized however that so far the estimation of the net driving force over the capillary membrane have only been based on determinations (at least qualitatively) of the intravascular oncotic and hydrostatic pressure conditions For a more valid estimation the corresponding parameters within the renal interstitial space have to be added In a previous report (Wunderlich *et al* 1971) a method was presented allowing access to the renal interstitium in which 50 μm thick PVC catheters were put under the renal capsule into the ubcapsular space This space is from anatomical point of view a part of the renal interstitium (that sur rounding the superficial and thereby acce sible tubules) Moreover it is in free com munication with the deeper parts of the renal interstitium as guided by the rapid appearance in the hilar lymph of labelled albumin infused into the subcapsular space The pre ent paper analyses the hydrostatic and oncotic pressure conditions on the two sides of the peritubular capillary membrane with the aim of (1) determining the net driving force over the membrane during both antidiuresis and extracellular volume expansion with isotonic saline and (2) describing a model for the regulation of the aforementioned forces in the renal interstitium

Material and methods

The experiments were performed on male Sprague Dawley rats we ghing between 270 g and 370 g under Inactin anesthes a administered i p with a dosage of 120 mg/kg The rats were tracheotomi ed and kept at 38 C by a servocontrolled heating pad The left femoral artery and vein were catheteriz d using indwelling polyethylene (PE) catheters The left kidney was exposed via a subcostal flank incision and suspended in a lu te cup The urether was can nulated clo e to the renal pelvis using as a rule s l con catheters The kidney surface was bathed with heated mineral oil or (when no acces to the kid y surfa e was needed) overed by rice paper drenched with o l

For technical reasons the different variables determin ng the dri ng forces o the peri tubular capillary membrane were studied in parate e periments as follow

Oncotic press res The oncotic pressure on the two des (int a ascular interstitial) of the capillary memb ane was investigated on 13 ts together w th studies on effect e renal pla ma flow (PAH-clearance) glomerular f ltration rate (Inulin cl arance) and urinary ex retory ar ables (urine flow sodium and potas um excretion

The oncotic pressure in the int rst t um and in the capillaries was calculat d f om protein data u in the formulas given by Land and Pappenheimer 1963 Th intra ascular protein concentration in the eno end of th peritubula capillaries was then put qual to the

systemic plasma concentration and that in the arterial end was calculated by dividing the systemic plasma concentration by a factor of (1—filtration fraction)

The interstitial protein concentration was estimated from samples of 1) interstitial fluid and 2) hilar lymph For interstitial sampling the subcapsular interstitial space was cannulated with about 50 μm thick PVC catheters For this purpose a hole was first made in the renal capsule With a glass instrument having a spherical tip of about 50 μm a 1 mm long subcapsular channel free from connective tissue bridges was formed The catheters (1—3) were then introduced (filled with coloured castor oil) into this channel and the whole was sealed off using a polymerizing silicon rubber (Elastosil 50 Wacker Chemie GMbH West Germany) The dissection and cannulation could usually be performed without any bleeding or visible deterioration of the tubules The sampling was carried out by gentle suction at a rate of 0 5—2 nl/min for 15 min. For lymph collection a hilar lymph vessel located in the region above the renal artery was dissected free and cannulated with PE catheters having inner diameters of more than 200 μm Smaller dimensions for these catheters were found to be unsuitable due to their high flow resistance In an appropriate collection condition the lymph vessel should be somewhat collapsed proximal to the catheter tip When the size of the lymph vessels was less than that of the catheters used, the vessels could still be cannulated due to their high distensibility The cannulation was then performed however using catheters cut into a sharp angle To prevent coagulation the catheters were filled with a 2 % Heparin solution prior to the insertion

The protein analyses were carried out according to Lowry *et al* (1951) and were adopted for microanalyses where the interstitial samples were concerned allowing determinations of 5 10^{-8} g protein or less The sample volume was measured as the length of the fluid column in a commercially available constant bore glass capillary (Microcups 0 5 μl Drummond Scientific Co USA) The final solutions (about 25 μl) were read at 50 mμ in a Beckman DU using microcuvettes as according to Klotz (Institute of Physiology Munchen West Germany)

For clearance studies PAH and 14 C Inulin were infused continuously Urine and blood were withdrawn at regular intervals—as a rule during half hour intervals The chemical analyses of PAH were determined according to Brun (1951) and the radioactive analyses by liquid scintillation counting Sodium and potassium concentrations were determined by flame photometry

Hydrostatic pressure measurements This series comprised an investigation on 6 rats carried out with a servo-controlled counter pressure device based on the principles outlined by Wiederhielm *et al* (1964) as modified by Intaglietta *et al* (1970)

For measurement of intracapillary pressure sharpened micropipettes with tip diameters ranging from 2—5 μm were used and were connected to a servocontrolled pump system filled with 0 5 M NaCl solution At a certain pressure the interface between the hypertonic saline and plasma (derived from the vessel punctured) was adjusted to be located at a certain level within the tip of the micropipette An increase in intravascular pressure would then move the interface upwards in the cannula causing an increased electrical resistance between the measuring system and the animal The increased resistance was then signaled to the associated electronics and gave rise to (by a pump) an increased pressure within the system until the resistance was restored The intravascular pressure was then measured as the pressure within the entire system and was recorded by a strain gauge transducer (Statham) Accurate pressures were obtained when the micropipette was located in the center of the punctured tubule or capillary Touching of the wall was found to lead to large and rapid alterations of the pressure Zero pressure was measured as the pressure recorded when the micropipette was suspended in the thin surface film between the kidney surface and the oil bathing the kidney

End efferent arteriolar pressures were taken as the pressures in the "star" vessels found on the surface of the kidney The pressure within the peritubular capillary network was estimated from the pressures obtained in third or fourth order branches For reference the proximal tubular pressure was also determined

The interstitial pressure was determined on 10 rats using the same counter pressure device as described in the preceding In these experiments however the pressure was recorded via the small PVC catheters placed in the subcapsular interstitial space The determinations were considered to be accurate when the sealing of the hole in the capsule resulted in a prompt rise in the pressure and when a high pressure was recorded when the interstitial pressure was raised by renal vein constriction or uretheral blockade 2 other experiments were discarded because of leakage when the pressure was raised in this manner

In 3 additional experiments the *albumin globulin* ratio in plasma and lymph was measured using gel-electrophoresis

Experimental conditions Two conditions were analyzed primarily viz 1) a control anti-diuretic condition with a continuous infusion of saline at a rate of 1 0—1 5 ml/h and 2) extracellular volume expansion here evoked by a slug injection of 5 ml saline and followed by a continuous injection at a rate of 8—12 ml/h

TABLE I Data from 13 rats on effective plasma flow (C PAH) glomerular filtration rate (C-Inulin) central hematocrit (HCT) urine excretory variables and on the protein concentration in plasma lymph and subcapsular space the lymph flu d flow and the corresponding protein flux and the L/P ratios of protein and inul n All values are given as the mean ± S D

Cond tions	C-PAH ml/min	C-Inulin ml/min	F ltration fract on	Hct	Urine flow ml/min 10³	Na excret on µM/min	K excretion µM/min
Control	4 74±1 10	1 19±0 23	0 26±0 06	46 2±4 2	2 67±0 97	0 28±0 25	p 79±0 30
Expansion	6 59±0 40	1 59±0 40	0 26±0 09	39 4±4 1	17 9±5 6	5 3±2 2	1 47±0 43
Change	+39 0	+33 6	±0	−15 6	+583	+189 3	+86 1

	Plasma prote n g	Lymph protein g	Lymph flow µl min	Lymph protein flux µg/m n	Sub-capsular protein g	L/P Protein	L/P Inulin
Control	6 06±0 95	1 74±0 71	1 35±1 11	17 0±9 9	1 49±0 97	0 30±0 12	0 68±0 16
Expansion	4 71±0 56	0 65±0 43	5 31±4 52	21 3±12 7	0 32±0 19	0 15±0 09	0 69±0 17
Change	−22 3	−62 6	+293	+25 3	−78 5	−50 0	+1 5

Results

In Table I the data on effective plasma flow glomerular filtration rate and urine excretory variables are presented together with data on protein concentrations in plasma lymph and interstitial fluid The figures fram each rat during control and extracellular volume expansion were derived from three or four determinations in each period

From Table I it would seem that the plasma flow increased from 4 7 to 6 6 ml/min and the glomerular filtration rate from 1 19 to 1 59 ml/min thus an increase of the same order of magnitude The filtration fraction thereby remained essentially unchanged The urine flow increased nearly tenfold with an even more marked rise in sodium excretion The plasma protein concentration decreased at the same time with about 20 % from the control value of 6 1 % The hematocrit was reduced to the same extent

The subcapsular interstitial protein concentration was estimated at 1 5 % during antidiuretic condition but fell drastically to values of about a few tenths of a percent during saline expansion The lymph protein data showed values in relatively good accordance with the interstitial protein values The lymph flow increased considerably during saline expansion whereas the lymph protein flow remained essentially unchanged estimated at 17 0 µg/min and 21 3 µg/min during antidiuresis and extracellular volume expansion respectively The difference is however hardly statistically significant (0 1 < P < 0 5)

The albumin globulin ratio in plasma as studied on 3 rats was estimated at 1 1 In lymph this ratio was considerably higher—estimated at 1 8 during both

TABLE II Hydrostatic pressure in the proximal tubules star vessels and peritubular capillaries of 6 rats and the interstitial pressure in 10 rats All values refer to mean ± S D

Condition	Proximal tubular pressure mm Hg	Star vessel mm Hg	Capillaries mm Hg	Interstitium mm Hg
Control	11 7 ± 1 0	14 1 ± 1 2	11 1 ± 1 1	1 32 ± 0 58
Expansion	14 9 ± 2 8	14 9 ± 2 5	12 2 ± 2 3	2 18 ± 1 22
% Change	+27 4	+5 7	+9 9	+65
	P < 0 05	P > 0 1	P > 0 1	P < 0 05

diuresis and extracellular volume expansion The albumin gamma globulin ratio was in plasma 3 8 and in lymph 7 7

The pressure in the proximal tubules the star vessels and the peritubular capillaries of 6 rats are shown in Table II (the pressure from each rat is derived from 3—10 single tubular and capillary pressure determinations which were not found to differ from each other by more than 1—2 mm Hg) In Table II the pressures measured in the subcapsular space in 10 rats have also been added

The peritubular capillary pressure was estimated at 11 1 mm Hg during antidiuresis showing a slight increase during extracellular volume expansion In the subcapsular interstitium the pressure increased almost twofold from 1 3 to 2 2 mm Hg during saline expansion even though the increase in absolute terms is very slight This was accompanied by a clearly visible increase in the size of the kidney

The intravascular pressure in the peritubular capillaries found here is somewhat higher than the pressures reported by Falchuk *et al* (1971) being less than 10 cm of H O The interstitial hydrostatic pressure is on the contrary much less than that *reported earlier using the needle pressure techniques (see Wunderlich et al* 1971)

From the data in Table II the hydrostatic pressure difference across the peritubular capillary membrane was calculated to be about 10 mm Hg both during antidiuresis and extracellular volume expansion The oncotic pressure in the renal interstitium was 4 0 and 0 8 mm Hg under the two conditions studied as calculated from the protein data and the albumin globulin ratio using equations given by Landis and Pappenheimer (1963)

The intravascular oncotic pressure was estimated at 27 7 mm Hg during antidiuresis decreasing to 19 0 mm Hg during saline expansion A summary of the driving forces is given in Fig 1

Discussion

In the overall judgement of the data obtained in this study it ought to be emphasized that the validity of all the parameters determined can be questioned

The interstitial pressures obtained might thus not be truly representative for the whole of the renal interstitium The previous finding (Wunderlich *et al* 1971) that

Pressur mm Hg	CONTROL		EXPANSION	
	Int t tium	Cap ll y	Inte st tium	C pilla y
O cot	4 0	27 7	0 8	19 0
Hyd ostat c	1 3	11 1	2 2	12 2
Pressur d ff ce				
On ot c	⟵ 23 7 ⟶		⟶ 18 2	
Hyd ost t	⟵ 9 8		⟵ 10 0	
N t	⟶ 13 9		⟶ 8 2	

Fig 1 Summary of driving forces operative over the peri tubular capillary membrane during control antidiuretic and extracellular volume expansion conditions

the subcapsular fluid was in free communication with the rest of the interstitium do however suggest that the pressures measured are correct at least with respect to order of magnitude This statement is furthermore supported by the finding (un published observations) that distal tubular pressure amounts to about 5 mm Hg The interstitial pressure will then probably be less than this value

The interstitial oncotic pressure might represent an underestimation as the presence of high molecular weight polysaccarides in the interstitium could cause a higher "effective oncotic pressure than that predicted from the protein concentra tion in the fluid derived from the interstitium (Laurent 1970)

The estimations of the intracapillary hydrostatic pressure is probably more correct Obstruction of the capillary punctured will presuambly not cause any pronounced error since the peritubular capillaries constitute a very richly anastomizing network The intravascular oncotic pressure is on the contrary subject to criticism since the fluid reab *orption rate as a function of capillary length remains unknown This* means that the protein concentration along the capillary and thereby the oncotic pressure obviously cannot be determined with a high degree of accuracy

Keeping the assumptions inherent in the different calculations in mind the fol lowing conclusions can be made

The amount of fluid reab orbed over a capillary membrane will be governed by the hydrostatic and oncotic pressure differences operating over the membrane ac cording to

$$J_v = \frac{Ap \times r}{\triangle x \times 8 \times \eta} \left(\bar{\sigma} \triangle \pi - \triangle P \right) \quad \text{where} \qquad (1)$$

J is fluid reabsorption Ap the pore area and r the pore radius $\triangle x$ the length of the pore η the viscosity of the fluid reabsorbed $\bar{\sigma}$ the mean reflection coefficient for the plasma protein $\triangle \pi$ the oncotic pre sure difference and $\triangle P$ the corresponding hydro- static pressure diference From this equation the total pore area per unit path length ($Ap/\triangle x$) for different pore radii can be calculated (Fig 2) η is then put to 0 007 poise (Landis and Pappenheimer 1963) and the $\bar{\sigma}$-values are taken from the data of

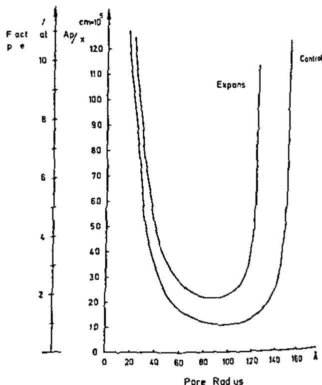


Fig 2 Predicted relationship between capillary pore area per unit path length $A_p/\Delta x$ and mean pore radii as calculated from the flow of fluid reabsorbed over the capillary membrane and the driving forces operative over this membrane utilizing the Poisseuilles formula The corresponding fractional pore area (seen in the ordinate scale to the left) is estimated from $A_p/\Delta x$ values assuming a pore length Δx of 0 3 mμ and using the figure of 350 cm² capillary surface area per gram kidney *as found by Kugelgen 1962* Note that the predicted pore area for large pore radii increases towards infinity This is due to the decrease in the reflexion coefficient for plasma proteins in these large pores causing a decreased effective colloid osmotic pressure

Durbin (1960) relating the reflection coefficient to the quotient between the molecule radius and the pore radius The molecule radius is put to 40 Å thus mainly corresponding to albumin (35 5 Å) since it is the chief source of oncotic pressure in plasma and interstitium

Assuming an effective pore length of 0 3 μm (Landis and Pappenheimer 1963) and the figure of 350 cm²/g cortical parenchyma for the total capillary area given for dog by Kugelgren and Braunger (1962) the fractional pore area can be calculated (see Fig 2) Within the pore radius range between 30 and 150 Å the fractional pore area is estimated at about 5 % or less At pore radii beyond this range the effective pore area increases towards infinity It can also be seen in Fig 2 that the effective pore area increases during saline expansion or (which seems more likely) the pores widen somewhat under this condition

The pore area estimated above is thus very large in comparison to the pore areas in capillaries in other vascular beds generally assumed to be in the order of one per mille It will allow a considerable leakage of plasma proteins unless the passage is highly restricted The degree of restriction *needed* to explain the observed rate of protein in the lymph can be calculated by the following equation

$$J_s = -Ap \times D \times \alpha \times dC(x)/dx - J_v \times \alpha \times C(x) \qquad (2)$$

where J_s is the net solute flow D the diffusion coefficient (6 24 and 4 24 10^{-7} cm/

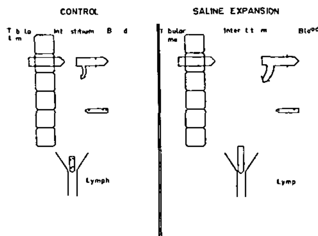


Fig 3 Hypothetical model describing the formation of interstitial fluid The fluid is assumed to be composed of 1) tubular reabsorbate escaping capillary reabsorption and 2) a protein containing solution derived from the plasma via large pores In saline expansion the capillary fluid reabsorption will be acutely diminished leading to a larger contribution of the colloid free part of the interstitial inflow and thereby to an increase in volume and hydrostatic pressure in the interstitium The amount of protein added will on the contrary remain essentially unchanged which then means that the interstitial protein concentration will decrease

sec for albumin and gamma globulin at 37° C respectively) α a conductance* factor assumed (see Forster 1971) to be valid both for diffusion and bulk flow transport and C(x) the concentration as a function of the pore length x Integrating this expression between x = 0 (i e the plasma side) and x = Δx (interstitial side) gives

$$J_s = -J_v \times \frac{C(x)_{x=\Delta x} - C(x)_{x=0}\ \exp - J_v \Delta x/ApD}{1 - \exp - J_v \Delta x/ApD} \quad (3)$$

The albumin flow has been estimated from turn over data of albumin (Kallskog and Wolgast forthcoming) to about 0 5×10⁻⁴ g/min This value also agrees on the whole with the lymph protein flow values found on higher mammals (see Mayerson 1963) Utilizing this value and the factor Ap/Δx for different radii calculated according to Eq 1 a J_v of 1 ml/min and the data on albumin concentration in plasma and interstitium (or lymph) respectively the conductance factor α will have a value of 10^{-4} or less Applying the equation for restricted diffusion by Renkin (1954) such a conductance is then compatible with a pore radius very close to that of the penetrating molecule In the case of albumin the pore radius should be 40 Å or less Pores of this size should not allow any transport of the gamma globulins for instance which is inconsistent with the finding of an albumin/gamma globulin ratio of 7 7 in lymph in relation to the figure of 3 8 in systemic plasma

It is therefore concluded that the peritubular capillary membrane is made up of (1) a number of small pores which do not allow leakage of plasma proteins and through which the main fluid reabsorption takes place and (2) some larger pores

* Varies from 0 to 1 when the restriction varies from complete impermeability to no hindrance of the solute transport

through which the proteins are added to the interstitium and to the lymph The proteins are here transported as a consequence of both diffusion and bulk flow in accordance with Eq 3

As the transport of two plasma proteins is known (albumin and gamma globulin having effective radii of about 35 5 Å and 50 Å respectively), two equations are obtained The exponent contains $J_r \Delta x/Ap$ which is determined by the net operative driving force and the square of the radius of the pores (see Eq 1) By dividing the two equations with each other in the case of a series of assumed pore radii the quotient between the a factor for gamma globulin and albumin for the different assumed pore radii can be calculated At 175 Å this quotient was found to fit with that predicted from Renkin equation (Renkin 1954) or in other words the radii of the large pores are about 150—200 Å In these pores there will be a negligible bulk flow as the effective oncotic pressure difference will balance the corresponding hydrostatic pressure difference The total pore area is comparatively small the factor $Ap/\Delta x$ estimated at 80 cm (The discussion is obviously based on the membrane constituting a porous membrane furthermore the pore radii calculated refer to the mean equivalent pore radii)

The experimental set up with the analysis of only two serum proteins identified obviously does not allow a more precise description of the peritubular capillary membrane of the rat kidney but the results are nevertheless suggestive for the pattern outlined with small and large pores as shown in Fig 3 According to this model some part of the tubular reabsorbate will escape capillary reabsorption and be added to interstitial and lymph fluids The proteins are added via the large pores

In saline expansion the intravascular oncotic pressure is reduced leading to an acute imbalance between tubular and capillary reabsorption and more of the tubular reabsorbate will escape capillary reabsorption and thereby add to the interstitium The protein flow out of the capillaries may remain essentially unchanged as found in the present investigation because the reduced plasma protein concentration (and subsequent lesser leakage) will be balanced by the increased transport caused by an increased outward directed bulk flow The interstitial pressure and volume will then increase and the interstitial protein concentration will decrease thus in accordance with the findings in the present investigation The sequence of events outlined will then act in the direction of a restoration of the driving forces although not entirely as was found in this paper It would therefore seem that the effective capillary reabsorption area increases during saline expansion probably due to a slight expansion of the pores (a 30 Å pore could expand to 37 Å)

The experimental set up does not permit the analyses of proximal tubular reabsorption Adopting the data given by Brenner and Berliner (1969) valid at conditions representing similar degrees of expansion (group IV) the fractional fluid reabsorption was reduced to some 60 % of the values in antidiuresis and the absolute reabsorption with 15—29 % The values for total tubular reabsorption in this study were in the same order of magnitude as in the present investigation and were interpreted as enhanced distal reabsorption (due to the increased load) partly compen

sating the deficient proximal reabsorption It is now suggested that the proximal fluid (and sodium) reabsorption by some as yet unidentified mechanism is determined by the net driving forces operating over the peritubular capillary membrane in the sense that acute changes of these forces will change the conditions within the renal interstitium with respect to volume and to hydrostatic and oncotic forces The change is in the direction that the net driving force over the peritubular capillary membrane will be restituted again

References

BANK N K M KOCK H S AYNEDJIAN and M ARAS Effect of changes in renal perfusion pressure on the supression of proximal tubular sodium reabsorption due to saline loading *J cl n Invest* 1969 *48* 271—283

BRENNER B and R W BERLINER Relationship between extracellular volume and fluid reabsorption by the rat nephron *Amer J Physiol* 1969 *217* 6—12

BRENNER B J L TROY and T M DAUGHARTY On the mechanism of inhibition in fluid reabsorption by the renal proximal tubules of the volume expanded rat *J clin Invest* 1971 *50* 1776—1780

BRUN C A rapid method for the determination of para aminohippuric acid in kidney function tests *J Lab clin Med* 1951 *37* 395

DAUGHARTY T M L J BELLEAU and L E EARLEY Interrelationship of physical factors affecting sodium reabsorption in dog *Amer Physiol* 1968 *215* 1442—1447

DAUGHARTY T M I F UEKI D P NICHOLAS and B BRENNER Comparative renal effects of isoncotic and coloid free volume expansion in the rat *Amer Physiol* 1972 *222* 225—235

DURBIN R P Osmotic flow of water across permeable cellulose membranes *J gen Physiol* 1960 *44* 315—326

FALCHUK K H B BRENNER M TADOKORO and R W BERLINER Oncotic and hydrostatic pressures in peritubular capillaries and fluid reabsorption by the proximal tubules *Amer J Physiol* 1971 *220* 1427—1433

FORSTER R E The transport of water in erythrocytes In *Current topics in membranes end transport* Ed F Bronner and A Kleinzeller Acad Press Inc New York 1971 ? 41—98

INTAGLIETTA M R F PAWULA and W R. TOMPKINS Pressure measurements in the mammalian microvasculature *Microvasc Res* 1970 2 212—220

KUGELGEN A and B BRAUNGER Quantitative Untersuchungen uber Kapillaren und Tubuli der Hundeniere *Z Zellforsch* 1962 *57* 766—778

LANDIS E M and PAPPENHEIMER, Exchange of substances through the capillary wall *Handbook of Physiology Circulation II* Waverly Press USA 1963 961—1034

LEWY J E and E WINDHAGER Peritubular control of proximal tubular reabsorption in the rat kidney *Amer Physiol* 1968 *214* 943—954

LAURENT T L The structure and function of intercellular Polysaccharides in connective tissue In Capillary Permeability Ed C Crone and N A Lassen Munksgaard Copenhagen 1970 261—277

LOWRY O H N J ROSEBROUGH A L FARR and R. J RANDALL Protein measurement with the Folin phenol reagent *J biol Chem* 1951 *193* 265—275

MAYERSON H S The physiologic importance of lymph *Handbook of Physiology Circulation II* Waverly Press USA 1963 1035—1073

RENKIN E M Filtration diffusion and molecular sieving through porous cellulose membranes *J gen Physiol* 1954 *38* 225—243

SPITZER A and E WINDHAGER Effect of peritubular oncotic pressure changes on proximal tubular fluid reabsorption *Amer J Physiol* 1970 *218* 1188—1193

WIEDERHIELM C A J W WOODBURY S KIRK and R. F RUSHMER Pulsatile pressures in the microcirculation of frog's mesentary *Amer J Physiol* 1964 *207* 173—176

WUNDERLICH P E PERSSON J SCHNERMANN H ULFENDAHL and M WOLGAST Hydrostatic pressure in the subcapsular interstitial space of rat and dog kidney *Pfl gers Arch ges Physiol* 1971 *328* 307—319

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From the Department of Physiology University of Turku Finland

Arginase Activity in Rat Small Intestinal Mucosa

By

M P HARRI and K HARTIALA

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Abstract

HARRI M P and K HARTIALA *Arginase activity in rat small intestinal mucosa* Acta physiol scand 1973 *89* 126—129

When the arginase activity of rat small intestinal mucosa was compared with that of the liver it was found to be 5 to 10 per cent to 1 g of tissue The mucosa of the glandular part of the stomach showed almost no arginase activity The intestinal activity was highest in jejunal mucosa and as a whole higher in male than in female rats

It is now well known that several conjugation reactions may occur in the intestinal mucosa for instance glucuronide formation sulphatation and esterification Non conjugative metabolic reactions are also found in the mucosa The formation of urea from arginine by intestinal arginase is an example of intestinal deconjugation reactions

Small amounts of arginase activity have been demonstrated in the small intestinal mucosa of dog (Kossel and Dakin 1904 Edlbacher and Bonem 1925) cat and probably also pigeon (Edlbacher and Bonem 1925) rabbit (Roche Mourgue and Baret 1953) and mouse (Greenstein and Meister 1952) Arginase activity was recently found in rat small intestine by Greengard Sahib and Knox 1970

In this study the duodenal jejunal and ileal mucosal arginase activities of rat are compared with those of the liver and the mucosa of the glandular part of the stomach

Materials and Methods

The experimental animals were 6 months old Wistar rats The females weighed 180 g to 240 g and the males 200 g to 360 g They were fed *ad libitum* with standard food pellets but fasted before the experiments for one day and night

The animals were killed by a blow on the neck and the tissue specimens were removed The stomach and the small intestine were opened and rinsed with ice cold buffer solution containing 0 067 M KH_2PO_4—$NaHPO_4$ 1 0 mM EDTA and 0 25 M sucrose pH 7 4 The mucosal specimens were removed from the glandular part of the stomach and small intestinal serosa with a knife and homogenized as were the liver specimen in the buffer solution using a glass homogenizer with a rotating Teflon pestle

TABLE I The arginase activities of rat small intestinal mucosa (duodenum jejunum ileum) compared with those of the liver and of the glandular part of the stomach

Tissue	Arginase activity	
	μmol of urea formed/min/g of tissue	S.E.
a) Females (7)		
Liver	158.0	8.3
Duodenal mucosa	9.4	1.3
Jejunal mucosa	13.2	1.5
Ileal mucosa	7.8	1.2
Glandular ventricular mucosa	0.2	0.1
b) Males (12)		
Liver	199.3	12.2
Duodenal mucosa	13.5	1.5
Jejunal mucosa	18.0	2.1
Ileal mucosa	11.6	1.0
Glandular ventricular mucosa	0.[illegible]	0.1

TABLE II Total urea synthesis of rat liver intestinal mucosa (duodenum jejunum ileum) and glandular ventricular mucosa calculated to the whole organ weights

Organ	Range of arginase activity μmol of urea/min/whole organ
a) Females (7)	
Liver	998–1260
Duodenal mucosa	1.7–5.3
Jejunal mucosa	10.8–32.7
Ileal mucosa	5.9–25.1
Small intestinal mucosa (total)	20.5–60.1
Glandular ventricular mucosa	0.0–0.3
b) Males (12)	
Liver	849–2400
Duodenal mucosa	1.6–8.4
Jejunal mucosa	7.4–43.[illegible]
Ileal mucosa	4.1–23.3
Small intestinal mucosa (total)	19.0–73.8
Glandular ventricular mucosa	0.1–0.6

The incubation mixtures contained 4.2 mg of arginine dihydrate (20.0 mM) in 1 ml of the buffer solution. The amounts of the tissue were selected to give a linear correlation of reaction velocity *to the corresponding tissue wet weights. They were 2.0 mg of liver, 100 mg of* glandular ventricular mucosa and 10 mg of each mucosal specimen from the various parts of the small intestine. The incubations were carried out at 37 °C for 10 min.

The formation of urea was measured with a modified diacetyl monoxime thiosemicarbazide method presented by Coulombe and Favreau 1963. 0.1 ml samples of the incubation mixtures were added to 0.9 ml of 5 % trichloroacetic acid and the precipitated material was removed by centrifuging. 0.2 ml of the clear supernatant was transferred to a tube containing 5 ml of the reagent freshly prepared from 5 mg of diacetyl monoxime and 0.25 mg of thiosemicarbazide in 50 % phosphoric acid. The colour was developed by boiling the mixture in a water bath for a period of 20 min. The colour intensity of the cooled samples was determined with a Hitachi spectrophotometer at 530 nm against a sample taken at the beginning of the incubation just after adding the substrate.

In order to examine the possible urease activity of the samples incubations with added (0.2 mg/ml) and no arginine were also carried out.

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From the Department of Physiology and the Department of Surgery II University of Goteborg Sweden

Vascular Reactions in the Small Intestine of the Cat during Hemorrhage

By

ULF HAGLUND

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Abstract

HAGLUND U *Vascular reactions in the small intestine of the cat during hemorrhage* Acta physiol scand 1973 *89* 129—141

The reactions within the consecutive vascular sections of the cat small intestine during and after 2 5 h hemorrhagic hypovolemia was studied using a plethysmographic technique The cats were bled 20 or 35 per cent of their estimated total blood volume The animals bled 35 per cent were divided into a shock and a non shock group according to the level of blood pressure prior to retransfusion Upon bleeding a moderate increase in intestinal blood flow resistance was observed but during the latter half of hypovolemia flow resistance decreased markedly in the shocked animals and was not normalized upon retransfusion The capillary surface area available for exchange remained unchanged during the hypovolemia except in the "shocked cats where a small increase was recorded Tissue volume decreased rapidly upon bleeding due to a venoconstriction but late in the hypovolemia and after retransfusion a continuous tissue volume increase was often seen partly due to a certain "pooling of blood but mainly caused by transcapillary transfer of fluid A great part of this fluid was found in the intestinal lumen at postmortem examination which also revealed hemorrhagic lesions in the mucosa of most of the animals

In earlier studies on cats model experiments were performed to follow the intestinal vascular bed and its responsiveness to neurogenic influences during regional intestinal (Haglund and Lundgren 1972 a b) and general hemorrhagic hypotension (Haglund and Lundgren 1973) These studies demonstrated a pronounced reduction of intestinal flow resistance and of neurogenic vascular responsiveness during hypotension as well as a poor restitution in this vascular bed once the hypovolemic situation was eliminated

In the experiments where general hemorrhagic hypotension was induced an experimental model close to the Wiggers model was used *i e* the animal was bled into a pressure bottle to maintain a constant arterial blood pressure throughout the hypotensive period With this constant pressure technique the compensatory mechanisms of the animal are however effectively counteracted by the experimental model itself The hemodynamic reactions reported from such studies have therefore

been suggested to be caused rather by the experimental model *per se* than by the hypotension (*e g* Swan and Nelson 1971) For such reasons it seemed important to study the intestinal vascular bed also during a hemorrhagic hypovolemic situation that allows nervous and humoral compensatory mechanisms to exert their full action If qualitatively the same chain of events would take place in such an experimental situation as in the earlier performed model experiments the latter type of approach would greatly simplify the analysis of the vascular reactions in the complex hemorrhagic shock situation

Methods

Experiments were performed on 22 cats weighing 2—5 kg anesthetized i v with chloralose (50 mg/kg b w) after induction with ether The cats had been deprived of food for 12 h and had no obvious signs of intestinal infection

The technique used has been described in details earlier (Folkow *et al* 1963 Haglund and Lundgren 1972 a 1973) and below only a brief description is given A segment of the jejunum, usually weighing about 35 g was chosen for the experiment and the remainder of the intestines was extirpated together with the spleen and the greater omentum The nervous supply to the intestinal segment was left intact, as were the adrenals

After heparinization the femoral arteries were cannulated for blood pressure recording and for arterial blood sampling respectively The superior mesenteric vein draining the intestinal segment and its lymph nodes was cannulated and blood flow was recorded by a drop recorder unit. The jejunal segment and its lymph nodes was enclosed in a perspex plethysmograph (Haglund and Lundgren 1972 a) and venous outflow pressure was set to 10 mm Hg (13—14 cm H_2O)

In three experiments the changes in tissue and regional blood volume were followed by e concomitant use of plethysmography and an intravascular tracer (Haglund and Lundgren 1972 a) erythrocytes from the animal being labelled with $^{51}NaCrO_4$ (AB Atomenergi Studsvik Sweden) according to Owen s technique (1959) The γ-emission of this tracer was registered by a scintillation detector (Philips) so collimated as to mainly register radioactivity from the tissues within the plethysmograph

During the operative procedures a slow bicarbonate infusion (20 meq $NaHCO_3$ per 100 ml 10 glucose solution 0 1 ml/min was given i v in order to minimize the effect of the surgical trauma on the acid base balance Arterial pH and P_{CO} and intestinal venous pH were checked throughout most experiments and in some also arterial P_O An Astrup pH meter pH meter 27 with a microelectrode unit was used for the measurements Further 2 ml of a dextran solution 1/3 Rheomacrodex® and 2/3 Macrodex® Pharmacia, Sweden) was given every 30 min to diminish rheological disturbances

After finishing the operative procedures the animals were allowed to rest for 30 min to obtain a stead state in art rial blood pressu e and intestinal blood flow An isovolumetric state of the intestinal preparation wa always btained during this period After the control period about 20 % 6 animals or 35 % 16 animals) of the estimated total blood volume (5 5 per cent of the body weight Farnsworth *et al* 1960 Aarseth and Bo 1972) was shed into Pedatrol bags in a stepwise fashion lasting 5—7 min The bags containing the shed blood were stored in a refrigerator for about 2 5 h and then retransfused after warming to body temperature The animal were then studied for an ther 30 min In some experiments intestinal tissue specimens were taken immediately after death for histological examination as described by Ahren and Haglund 1973

Results

As described in Methods the animals were bled 20 % or 35 % respectively of their estimated total blood volume The results obtained in the two series below named moderate and "severe bleeding respectively will be described separately

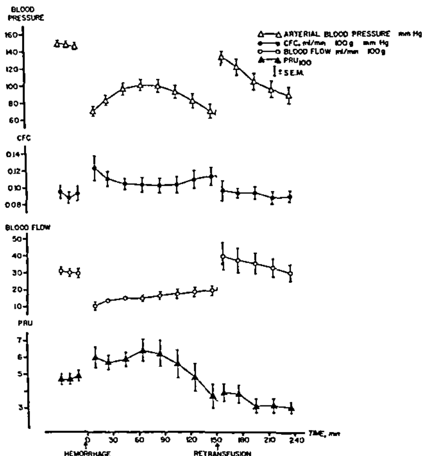


Fig 1 The effects of severe bleeding (35 % of the estimated total blood volume) on arterial blood pressure intestinal capillary filtration coefficient (CFC) blood flow and flow resistance (PRU_{100}) Each point represents mean value ± S E from 12 expts Lines drawn by inspection

A Severe bleeding

Here the 16 animals were bled approximately 35 per cent of their estimated total blood volume 4 cats died after 30—120 min of hypovolemia and they were therefore excluded from the material The average results from the remaining 12 are presented in Fig 1 and Table I Since response to hypovolemia varied among these cats they were divided into two groups according to the effet of hypovolemia on arterial blood pressure (see Fig 2 and 3) In one group (7 animals) arterial pressure fell below 70 mm Hg late in hypovolemia These cats are considered to be in shock and they are referred to as the shock series Two of these animals died during the post hypovolemic period In the remaining 5 animals the fall in arterial pressure was not that pronounced during bleeding and they were therefore labelled the non shock series all of them survived the posthypotensive control period

TABLE I Arterial and intestinal venous blood pH as well as arterial PCO_2 before, during and after a 2 5 h hypovolemia induced cy bleeding the animals 20 or 35 per cent of estimated total blood volume Mean values ± S E n = number of observations

Time			Control	Hypovolemia
Time min		n		5
Hemorrhage 20 %	Arterial blood pH	6	7 32±0 03	7 41±0 04
	Intest venous blood pH	6	7 26±0 02	7 24±0 03
Hemorrhage 35	Arterial blood PCO_2	7	35±3	24±2
	Arterial blood pH	12	7 33±0 01	7 44±0 0?
	Intest venous blood pH	12	7 29±0 01	7 24±0 01

1 Mean arterial pressure From a resting value of 146±4 mm Hg (mean±S E n=12) arterial pressure fell to 70±5 mm Hg 10 min after the bleeding (Fig 1) During the first 60—90 min of hypovolemia (compensation period) blood pressure increased slowly to about 100 mm Hg after which it fell gradually to 69±8 mm Hg during the remaining 60—90 min (decompensation period) before retransfusion was performed Retransfusion led to a rapid return towards the control pressure level but during the subsequent normovolemic period blood pressure decreased continuously

As stated above the level of the arterial pressure at the end of the hypovolemic riod formed the basis for dividing the animals into two series Fig 2 illustrates the different blood pressure levels in the two series throughout the experimental procedures In the shock series a much more pronounced blood pressure fall occurs during the phase of decompensation as well as during the posthypotensive control period

2 Intestinal resistance vessels The effects of hemorrhage on intestinal blood flow and flow resistance are shown on Fig 1 2 and 3 Fig 1 illustrates the average response pattern of both the shock and non shock animals while that of the shock and non shock groups are compared to each other in Fig 2 and 3 In principle the changes in resistance are largely similar in all three figures Upon bleeding blood flow is reduced to about 1/3 of control followed by a continuous flow increase during hypovolemia It should be noted that blood flow during the hypovolemic period was if anything greater in the shock group than in the non shock group In the early compensation period of hemorrhage flow resistance was augmented in all animals but it fell gradually in the late decompensation period particularly in the shock group (Fig 2)

Upon retransfusion blood flow initially returned towards and above pre hemorrhagic control in both groups (Fig 3) as combined with a fall in flow resistance in the non shock group while resistance increased in the shock

		Control	
60	120	5	45
7.33±0.02	7.32±0.03	7.25±0.04	7.30±0.03
7.20±0.02	7.18±0.02	7.18±0.03	7.23±0.02
25±2	2[illegible]±2	26±3	31±3
7.34±0.02	7.32±0.03	7.17±0.05	7.23±0.04
7.18±0.03	7.16±0.03	7.11±0.04	7.19±0.03

group. During the subsequent period of normovolemia intestinal blood flow fell continuously in the "shock" animals to values slightly below prehypotensive control while it remained above control throughout in the "non-shock" animals. During the same period flow resistance decreased in the "shock" series while it was more or less constant in the other series.

3. Exchange vessels. The reactions of the precapillary "sphincters" as reflected in the alterations of the capillary filtration coefficient (CFC) differed in the two groups of animals (Fig. 3). CFC tended to stay above control during hypovolemia in the "shock" group while it was largely unaltered in the "non-shock" group. Upon retransfusion CFC reached control values in both groups.

4. Changes of tissue volume and regional blood volume (capacitance vessels). Upon bleeding intestinal volume rapidly decreased 3.5 ± 0.2 ml/100 g (mean ± S.E., n = 12). Most of this volume reduction occurred within 30 sec after the bleedings. To judge from those experiments in which blood and tissue volumes were concomitantly measured, the reduction of tissue volume was solely caused by a decrease of regional blood content, *i.e.* a consequence of constriction and/or passive elastic recoil of the capacitance vessels. In most animals no further change of tissue volume was registered until during the "decompensation" period, when tissue volume in 2 animals (1 "shock" and 1 "non-shock" animal) increased gradually about 0.1 ml per 100 g and min. Part of this volume augmentation, ultimately amounting to 6—8 ml/100 g, was caused by an increase of the intestinal blood content, since regional blood volume increased simultaneously 1—1.5 ml/100 g tissue during the final hour of hypovolemia. The remaining tissue volume augmentation corresponded to a capillary pressure elevation in the order of 1—3 mm Hg if it is assumed that it was solely due to transcapillary filtration.

After retransfusion blood volume rapidly returned to the prehypovolemic control level. During the subsequent normovolemic period tissue volume again increased progressively in 8 animals (4 "shock" and 4 "non-shock" animals), partly due to

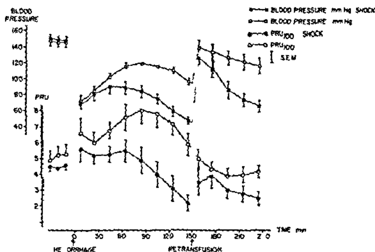


Fig 2 The effects of severe bleeding (35 % of the estimated total blood volume) on arterial blood pressure and intestinal flow resistance (PRU_{100}) in "shocked" (n = 7) and "non shocked" (n = 5) animals Lines drawn by inspection

further augmentation of regional blood content amounting to 0 5—1 ml/100 g during the first 15—30 min after retransfusion The additional increase in tissue volume during this normovolemic period must then by exclusion reflect an increase of tissue fluid Assuming that the single cause for this was a mean capillary pressure elevation such a pressure elevation would amount to 1—4 mm Hg

5 *Postmortem examination of the intestine* Fluid corresponding to 55—100 per cent of the calculated increase in tissue fluid volume was found in the intestinal lumen at the end of the experiments Further lesions in the intestinal mucosa

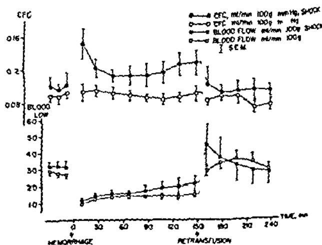


Fig 3 The effects of severe bleeding (35 % of the estimated total blood volume) on blood flow and capillary filtration coefficient of the small intestine in "shocked" (n = 7) and "non shocked" (n = 5) animals Lines drawn by inspection

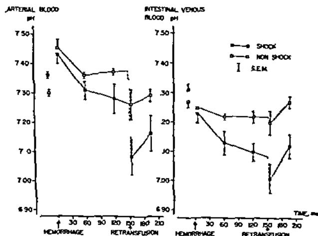


Fig 4 pH of arterial blood (left panel) and of intestinal venous blood (right panel) before during and after hemorrhagic hypovolemia induced by bleeding the animals 35 % of their estimated total blood volume The material was divided into a "shock (n = 7) and a non shocked (n = 5) group of animals as described in the text Lines drawn by inspection

varying from scattered petechial bleedings to large confluent areas of hemorrhagic necroses were regularly found Microscopic examination of the intestinal tissue samples revealed epithelial lifting and often denuded villi No clear difference was in these respects observed between the animals in the shock and non shock groups

6 *Blood pH and P_{CO_2} during hypovolemia* All animals received a slow bicarbonate infusion (see Methods) during the operative procedures while no means to stabilize acid base balance were taken during the actual experiment Arterial and intestinal venous blood pH and arterial P_{CO_2} were determined intermittently and the results are summarized in Table I

A comparison between the shock and non shock groups is illustrated in Fig 4 The changes induced were qualitatively similar but quantitatively quite different An initial transient increase of arterial pH was observed in both groups probably due to a reflex hyperventilation as reflected in a reduced arterial P_{CO_2} (Table I) However despite the fact that arterial as well as venous pH was higher in the shock animals compared with the non shock group during control the subsequent hypovolemia caused a more pronounced decrease of both arterial and intestinal venous pH in the shocked cats Moreover retransfusion revealed a much more pronounced hidden tissue acidosis in these animals as suggested by a marked further reduction of blood pH In addition blood pH never returned to control in the shock group

B Moderate bleeding

This series of experiments comprised six animals which were bled about 20 per cent of their estimated total blood volume Fig 5 illustrates the effects of this extent of blood loss which all these animals were able to survive on arterial blood pressure intestinal blood flow CFC and flow resistance (PRU_{100}) The changes in blood pH

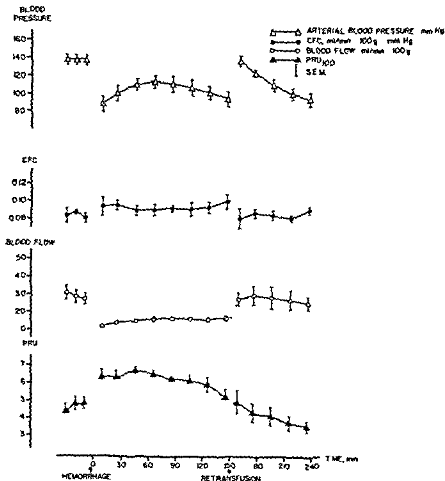


Fig 5 The effects of moderate bleeding (20 % of the estimated total blood volume) on arterial blood pressure intestinal capillary filtration coefficient (CFC) blood flow and flow resistance (PRU_{100}) Each point represents mean value ± S E from 6 expts Lines drawn by inspection

are given in Table I It is apparent from Fig 5 and Table I that the response pattern evoked by this moderate bleeding was qualitatively similar to that observed during severe bleeding although quantitatively less pronounced (cf Fig 1)

Upon bleeding tissue volume rapidly decreased 1 5 ± 0 2 ml/100 g (mean ± S E $n=6$) and within 3—5 min a new isovolumetric state was achieved that was maintained throughout the hypovolemia Upon retransfusion a rapid tissue volume augmentation occurred of largely the same magnitude as the decrease seen upon bleeding During the subsequent normovolemic period tissue volume increased slowly in 4 of the experiments at a rate of 0 15—0 20 ml/min × 100 g but it tended to level off after 45—60 min Fluid corresponding to 50—70 per cent of this volume increase was found accumulated in the intestinal lumen

Hemorrhagic lesions in the intestinal mucosa were regularly found at postmortem examination also in these animals However most often these lesions had the appearance of petechial bleedings and no signs of more extended lesions were observed No microscopic examination was performed in this series of experiments

Discussion

The present study constitutes part of a eries of experiments aimed at describing and analysing the reactions within the consecutive sections of the small intestinal vascular bed during hypotension and hemorrhagic shock In earlier publications the reactions in the denervated small intestine during prolonged regional hypotension were described (Haglund and Lundgren 1972 a) Further the competition between the remote nervous and the local metabolic vascular control was studied during regional and general hemorrhagic hypotension (Haglund and Lundgren 1972 b 1973) The present investigation—in which the reactions within the consecutive vascular sections of the intact small intestine were investigated during two levels of hemorrhagic hypovolemia—represents the final study of this series Here the compensatory mechanisms of the experimental animal were allowed to fully counter act the induced bleeding to the extent possible in an anesthetized animal and in an experimental model that as far as possible mimicks the hemorrhagic shock seen in clinical medicine

In the present study only those animals which exhibited an arterial blood pressure below 70 mm Hg during the last part of the hypovolemia were considered to be in shock and the following discussion will be concentrated on these animals In the non shock group of animals the same qualitative reactions were observed as in the shock group but a clear quantitative difference was obvious (Fig 1 2 3 5)

Most authors seem to be convinced that the changes in the peripheral vascular resistance occurring upon bleeding are predominantly initiated by a reflex increase in sympathico adrenal activity inducing a constriction of the vascular smooth muscle cells (see Chien 1967) For example bleeding causes a reflex intestinal vasoconstriction via the baroreceptors in cats (*e g* Öberg 1964 Kendrick *et al* 1972) and also in rabbits (Chalmers *et al* 1967) Further α receptor blockade reduces the intestinal vasoconstriction after hemorrhage in dogs (Lillehei *et al* 1964) The sympathetic rate of discharge seems to be largely unchanged throughout a hypovolemic period (Lundgren *et al* 1964) although a final decrease in the sympathetic firing rate might occur just prior to death (Lundgren *et al* 1964 Chien 1967) Therefore any failure of the sympathetic nervous system to maintain the control of the intestinal vascular bed is in the present discussion considered to be a result of local vaso dilator influences *i e* an accumulation of vasodilating metabolites as discussed at length by Lewis and Mellander (1962) and Haglund and Lundgren (1972 b) It should be pointed out in this connection that the sympathetic vascular control seems to be mainly exerted by the vasomotor nerves the release of catecholamines from the adrenals being of relatively minor importance (Celander 1954)

Upon bleeding intestinal flow resistance increased rapidly about 20 per cent above control in the shocked animals (Fig 2) However, after 60—90 min the resistance vessels began to dilate again and during the remaining period of the hypovolemia flow resistance fell continuously to values below half those of control (Fig 2 and 3) In all probability this reflects a progressive regional accumulation of dilating metabolites as was also suggested by the declining neurogenic responsiveness of the intestinal vessels during regional or general hypotension as described earlier (Haglund and Lundgren 1972 b 1973) In the non shocked group of animals on the other hand the intestinal flow resistance was kept elevated throughout the hypovolemia The reason for this difference between the two groups is not quite clear It is apparently not related to the blood flow level directly during hypovolemia since intestinal blood flow if anything was lower in the non shocked animals (Fig 3) One factor of importance may be the level of arterial blood pH in the two groups (Fig 4) since it was demonstrated in an earlier publication (Haglund and Lundgren 1973) that alkalosis increased the nervous responsiveness of the intestinal vessels An increased sensitivity to noradrenaline infusion was also exhibited during alkalosis by the perfused cat hind limb (Bygdeman 1963) It is evident from Fig 4 that the average pH of the non shocked group of animals was significantly higher than control probably due to a reflexly induced hyperventilation (Table I) This high blood pH will increase the vascular response to vasoconstrictor fibre activation and may consequently at least partly explain the mentioned difference

In agreement with findings of the earlier model experiments (Haglund and Lundgren 1972 a b 1973) the tone of the intestinal resistance vessels was never restored after retransfusion contrasting to what was reported for the skeletal muscle vascular bed by Mellander and Lewis (1963) These observations suggest that the control of the intestinal vascular smooth muscle become more severely damaged during hypovolemia

The response pattern of the intestinal capacitance vessels as reflected in changes in the regional blood volume was largely similar to that of the resistance vessels Upon severe bleeding an abrupt decrease of the regional blood content was recorded amounting to about 3 5 ml/100g This constriction of the capacitance vessels may be induced passively by a lowered transmural pressure during hypotension and/or by an active neurogenic constriction It seems likely that the passive component is smaller than the active one since earlier model experiments indicate that lowering arterial pressure to about 60 mm Hg (*cf* Fig 1) leads to only a small reduction of the regional blood content less than 1 0 ml/100 g (Haglund and Lundgren unpublished observations) This means that the increased vasoconstrictor fibre activation induced by the bleeding would account for at least two third of the reduction in blood content *i e* about 2 5 ml/100 g a value in close agreement with that earlier reported when electrically stimulating the regional sympathetic fibres at 4 or 8 Hz (Haglund and Lundgren 1972 b 1973) In the late phase of hypovolemia and during the posthypovolemic period an augmentation of the regional blood content was observed probably caused by the progressive accumulation of local metabolites

as suggested by earlier model experiments (Haglund and Lundgren 1972 b 1973) Thus these observations give support for some pooling of blood in the intestinal vascular bed during shock although such a factor alone cannot by far explain the development of shock in these animals the total amount of blood pooled in the intestine being only 1—2 ml in a 3 kg cat

The concomitant decline of nervous responsiveness of the intestinal resistance vessels (mainly localized to the precapillary side) and capacitance vessels (mainly localized to the postcapillary side) suggests that the pre /postcapillary resistance ratio and hence mean capillary pressure is hardly changed by the sympathetic neurogenic influence *per se* during hypovolemia This conclusion is also supported by observa tions made in earlier model experiments (Haglund and Lundgren 1972 b 1973) In this respect the intestinal vascular bed behaves differently from the skeletal muscle where the sympathetic constrictor fibre influence fails more on the pre capillary side than on the postcapillary one eventually to such an extent that capillary pressure rises again and may in some situations turn the normally occurring absorption into a filtration loss from the intravascular compartment (Lewis and Mellander 1962 Mellander and Lewis 1963 Lundgren *et al* 1964)

However quite apart from the extrinsic neurogenic influence an increase of the intestinal tissue fluid volume was observed also in the present study in the late phase of hypovolemia as well as during the posthypotensive period This was also seen in earlier model experiments with intermittent sympathetic stimulation and it occurred both during and between stimulation periods The cause of this tissue volume augmentation has been discussed earlier (Haglund and Lundgren 1972 a 1973) and secretion as well as an increased capillary permeability were less likely causes Two other mechanisms both disturbing the Starling equilibrium across the capillary wall seem pathogenetically possible (Haglund and Lundgren 1972 b 1973) 1 A derange ment of the intestinal autoregulation which seems to be mainly designed to keep mean capillary pressure constant despite variations in arterial blood pressure (Folkow *et al* 1963 Haglund and Lundgren 1972 a) 2 A local increase in the extravascular colloid osmotic pressure possibly due to the epithelial sloughing of cells as seen at the tips of the villi (Åhren and Haglund 1973)

The capillary filtration coefficient (CFC) reflecting in a semiquantitative manner the surface area available for porebound transcapillary exchange remained un changed (moderate bleeding and non shock group of severe bleeding) or slightly elevated above control (shock group) during hypovolemia Since it is well established that CFC becomes decreased during sympathetic activation (*cf* Haglund and Lundgren 1972 b 1973) this indicates that the neurogenic influence on the pre capillary sphincter smooth muscles was counteracted by dilating local metabolites as discussed above for the resistance and the capacitance vessel A similar train of events were recorded in earlier model experiments (Haglund and Lundgren 1972 b 1973)

At *post mort m* examination hemorrhagic mucosal lesions were found in the intestine of all animals exposed to severe blood loss while only scattered petechial

mucosal bleedings were observed after moderate bleedings The histological appearance of the hemorrhagic lesions of this study was similar to that reported earlier during regional hypotension (Åhren and Haglund 1973) The pathogenesis of these lesions was discussed at length in earlier publications (Haglund *et al* 1973 Haglund and Lundgren 1973) and it was suggested that hypoxia in the villous tips was produced by an increased effectiveness of the villous countercurrent exchanger (Lundgren 1967) as a result of the greatly decreased linear flow rate in the hairpin vascular loops of the villi It seems likely that the presently described lesions have largely the same pathogenesis

McNeil *et al* (1970) failing to find any effect of dibenzyline on the intestinal resistance vessels upon bleeding suggested that the sympathico adrenal system plays an insignificant role in explaining the intestinal resistance increase after hemorrhage and that instead vasopressin and angiotensin are of importance They performed experiments where intestinal blood flow changes in intact animals were compared to those registered in animals where intestinal denervation adrenalectomy and/or hypophysectomy and nephrectomy had been performed However the blood flow changes were expressed in an indirect way based on the implicit assumption that the pressure flow curve for the intestinal vascular bed remains unaltered during the different experimental procedures This assumption probably does not hold true when *e g* the sympathetic discharge to the intestine is increased as is illustrated *e g* by the changes in pressure flow relationship in the kidney when the vasoconstrictor fibres are activated (Folkow and Langston 1964) Such a possible influence makes their results difficult to evaluate Moreover the failure of an α blocking agent to feet intestinal flow resistance during hypovolemia may be an expression of a decreased vascular responsiveness to neurogenic influences as observed in this and previous studies

Both the resistance and capacitance responses seen upon bleeding closely mimick those found in earlier described model experiments (see above) which strongly suggests that nervous mechanisms are to a large extent responsible for the recorded hemodynamic response pattern It can however not be excluded that humoral mechanisms might contribute to the vasoconstriction found However since no vasoconstriction was recorded in experiments where the effect of hemorrhage on the denervated intestine was studied and further no difference in the terms of intestinal resistance vessel was registered when regional and hemorrhagic hypotension were compared (Haglund and Lundgren 1972 b 1973) the contribution of blood borne agents must be small Finally a tachyphylaxis to angiotension develops quite rapidly in the intestinal vascular bed (Jonsson *et al* 1967) and the direct capacitance response to this drug is rather small (Folkow *et al* 1961) Vasopressin on the other hand induces a marked and sustained resistance response in the intestinal vascular bed, but a very small capacitance one (Haglund and Svanvik to be published)

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References

AARSETH P and G BO Content of blood and of extravascular water in cat lungs during changes in total blood volume *Acta physiol scand* 1972 *85* 343—352

AHREN C and U HAGLUND Mucosal lesions in the small intestine of the cat during low flow *Acta physiol scand* 1973 *88* 541—550

BYGDEMAN S Vascular reactivity in cats during induced changes in the acidbase balance of the blood *Acta physiol scand* 1963 *61* Suppl 222

CELANDER, O The range of control exercised by the sympathetico-adrenal system *Acta physiol scand* 1954 Suppl. 116

CHALMERS J P P I KORNER and S W WHITE Effects of hemorrhage on the distribution of the peripheral blood flow in the rabbit. *J Physiol* (Lond) 1967 *192* 561—574

CHIEN S Role of the sympathetic nervous system in hemorrhage *Physiol Rev* 1967 *47* 214—288

FARNSWORTH P N C N PALLINO-GONZALES and M J GREGERSEN F_{cells} values in the normal and splenectomized cat Relation of F_{cells} to body size *Proc Soc exp Biol* (N Y) 1960 *104* 729—733

FOLKOW B and J LANGSTON The interrelationship of some factors influencing renal blood flow autoregulation *Acta physiol scand* 1964 *61* 165—176

FOLKOW B B JOHANSSON and S MELLANDER The comparative effects of angiotensin and noradrenaline on consecutive vascular reactions *Acta physiol scand* 1961 *53* 99—104

FOLKOW B O LUNDGREN and I WALLENTIN Studies on the relationship between flow resistance capillary filtration coefficient and regional blood volume in the intestine of the cat *Acta physiol scand* 1963 *57* 270—283

HAGLUND U and O LUNDGREN Reactions within consecutive vascular sections of the small intestine of the cat during prolonged hypotension *Acta physiol scand* 1972 a *84* 151—163

HAGLUND U and O LUNDGREN The effects of vasoconstrictor fibre stimulation on the consecutive vascular sections of the smal lintestine of the cat during prolonged regional hypotension *Acta physiol scand* 1972 b *85* 547—558

HAGLUND U and O LUNDGREN The effects on vasoconstrictor fibre stimulation on consecutive vascular sections of cat small intestine during hemorrhagic hypotens on *Acta physiol scand* 1973 *88* 95—108

HAGLUND U O LUNDGREN and J SVANVIK On the pathogenesis of the intestinal mucosal lesions in shock *Acta physiol scand* 1973 *87* 49 A—50 A

JONSSON O J SVANVIK and P VIKGREN Regional differences in vascular tachyphylaxis to angitension in the cat *Angiologica* 1967 *4* 299—309

KENDRICK E B ÖBERG and G WENNERGREN Vasoconstrictor fibre discharge to skeletal muscle kidney intestine and skin at varying levels of baroreceptor activity in the cat *Acta physiol scand* 1972 *85* 464—476

LEWIS D H and S MELLANDER, Competitive effects of sympathetic control and tissue metabolites on resistance and capacitance vessels and capillary filtration in skeletal muscle *Acta physiol scand* 1962 *56* 162—188

LILLEHEI R. C J K LONGERBEAM J H BLOCK and W G MANAX The nature of irre ersible shock *Amer Surg* 1964 *160* 682—708

LUNDGREN O Studies on blood flow distribution and ountercurrent ex hange in the small intestine *Acta physiol scand* 1967 Suppl 303

LUNDGREN O J LUNDVALL and S MELLANDER, Range of s mpathet c discharge and reflex vascular adjustments in skeletal muscle during hemo rhagic hypotension *Acta phys ol scand* 1964 62 380—390

MELLANDER S and D H LEWIS Effect of hemorrhagic shock on the reactivity of resistance and capacitance essels and on capillary filtration transfer n cat skeletal mu cle *C rculat R s* 1963 *8* 105—118

McNEIL R. J R. D STARK and C V GREENWAY Intest nal vasoconstriction after hemorrhage Roles of vasopress n and angiotensin *Amer J Phys ol* 1970 *219* 1342—1347

OBERG B Effects of cardio ascular reflexes on net capillary fluid transfer *A ta physiol scand* 1964 62 Suppl 229

OWEN C A *D agnost c Radioisotop s* Charles C Thomas Publ Springf ld Ill 1959

SWAN H and A W NELSON E per mental lethal can ne hemorrhage Ischem c adbomin l viscera absen e of hemorrhagic gut on portal congestion *J s rg R s* 1971 *11* 6

mucosal bleedings were observed after moderate bleedings The histological appearance of the hemorrhagic lesions of this study was similar to that reported earlier during regional hypotension (Åhren and Haglund 1973) The pathogenesis of these lesions was discussed at length in earlier publications (Haglund *et al* 1973 Haglund and Lundgren 1973) and it was suggested that hypoxia in the villous tips was produced by an increased effectiveness of the villous countercurrent exchanger (Lundgren 1967) as a result of the greatly decreased linear flow rate in the hairpin vascular loops of the villi It seems likely that the presently described lesions have largely the same pathogenesis

McNeil *et al* (1970) failing to find any effect of dibenzyline on the intestinal resistance vessels upon bleeding suggested that the sympathico-adrenal system plays an insignificant role in explaining the intestinal resistance increase after hemorrhage and that instead vasopressin and angiotensin are of importance They performed experiments where intestinal blood flow changes in intact animals were compared to those registered in animals where intestinal denervation adrenalectomy and/or hypophysectomy and nephrectomy had been performed However the blood flow changes were expressed in an indirect way based on the implicit a sumption that the pressure flow curve for the intestinal vascular bed remains unaltered during the different experimental procedures This assumption probably does not hold true when *e g* the sympathetic discharge to the intestine is increased as is illustrated *e g* by the changes in pressure flow relationship in the kidney when the vasoconstrictor fibres are activated (Folkow and Langston 1964) Such a possible influence makes their results difficult to evaluate Moreover the failure of an α blocking agent to ct intestinal flow resistance during hypovolemia may be an expression of a decreased vascular responsiveness to neurogenic influences as observed in this and previous studies

Both the resistance and capacitance responses seen upon bleeding closely mimick those found in earlier de cribed model experiments (see above) which strongly suggests that nervous mechanisms are to a large extent responsible for the recorded hemodynamic respon e pattern It can however not be excluded that humoral mechanisms might contribute to the va oconstriction found However since no vasoconstriction was recorded in experiments where the effect of hemorrhage on the denervated intestine was studied and further no difference in the terms of intestinal resistance vessel was registered when regional and hemorrhagic hypotension were compared (Haglund and Lundgren 1972 b 1973) the contribution of blood borne agents must be small Finally a tachyphylaxis to angiotension develops quite rapidly in the intestinal vascular bed (Jonsson *et al* 1967) and the direct capacitance response to this drug is rather small (Folkow *et al* 1961) Vasopressin on the other hand induces a marked and sustained resistance response in the intestinal vascular bed but a very small capacitance one (Haglund and Svanvik to be published)

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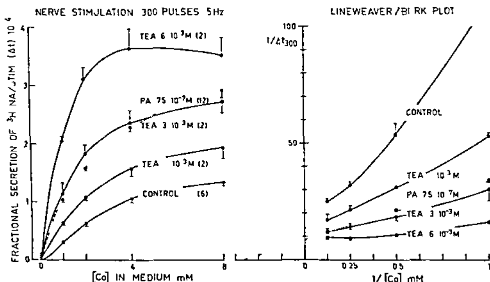


Fig 1 Left panel Average fractional secretion of ^{3}H NA/ stimulus (△t) as a function of [Ca] in medium Effect of PA 7 5×10⁻⁷ M (broken line) and of TEA 1 3 and 6 mM Right panel Double reciprocal plot of fractional secretion of ^{3}H NA/300 stimuli (△t300) against [Ca] in medium. Vertical bars S E (CONTROL and PA) or range (TEA) Brackets Number of observations

normetanephrine 10⁻⁵ M were added to prevent rebinding of NA and since the evoked rise in efflux of ^{3}H was completely blocked by tetrodotoxin 5×10⁻⁷ g/ml it is assumed that the evoked fractional rise in efflux of total ^{3}H

$$\frac{\text{evoked rise in efflux of } ^3\text{H}}{\text{total } ^3\text{H in tissue at the time of stimulation}}$$

gives a valid measure of the secretion of ^{3}H NA The Lineweaver Burk plot was constructed by substituting fractional secretion of ^{3}H NA for reaction velocity and calcium for substrate as previously described (Stjarne 1973 b)

The results are shown in Fig 1 As seen in the left panel the curve describing the dependence of ^{3}H NA secretion on external calcium in the absence of drugs interfering with the secretory mechanism (CONTROL) is sigmoid in shape Addition of PA 7 5×10⁻⁷ M shifts the curve to the left and transforms it into a rectangular hyperbola in the Lineweaver Burk double reciprocal plot of ^{3}H NA secretion against calcium in the medium a straight line is obtained This suggests that NA secretion in the absence of alpha adrenoceptor mediated negative feedback control is a simple function of external calcium (*cf* Stjarne 1973 b)

As also seen in Fig 1 TEA at the concentrations used 1—6 mM markedly and dose dependently enhances ^{3}H NA secretion However the shape of the curves describing the calcium dependence of ^{3}H NA secretion in the presence of different concentrations of TEA is quite different from that of those obtained with PA (Fig 1

cf Stjarne 1973 b) While the curve at PA 7.5×10^{-7} M (left panel) and the corresponding straight line in the Lineweaver Burk plot (right panel) indicate that release from alpha adrenoceptor mediated negative feedback control has made ^{3}H NA secretion completely dependent on external calcium throughout the range used the secretory maximum is reached already at calcium 2 mM in the presence of TEA mM Interestingly this level of ^{3}H NA secretion coincides fairly well with the V_{max} for ^{3}H NA secretion (intercept with the ordinate in the Lineweaver Burk plot) in the presence of *PA 7.5×10^{-7} M*

While the induced alterations in ^{3}H NA secretion may in both cases involve modulation of availability of external calcium for the secretory mechanism the present results do not suggest that the opposite effects of TEA, which enhances ^{3}H NA secretion and NA which depresses this function (Starke 1972 Stjarne 1973 c) are due to an antagonistic action on the same functional target

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References

ARMSTRONG C M and I BINSTOCK Anomalous rectification in the squid giant axon injected with tetraethyl ammonium chloride *J gen Physiol* 1965 *48* 856—872

JENKINSON D H and I K M MORTON Effects of noradrenaline and isoprenaline on the permeability of depolarized intestinal smooth muscle to inorganic ions *Nature* (Lond) 1965 *205* 505—506

KATZ B and R MIDELI Tetrodotoxin resistant electric activity in presynaptic terminals *J Physiol* (Lond) 1969 *203* 459—487

KIRPEKAR, S M J C PRAT M PUIG and A R WAKADE Modification of the evoked release of noradrenaline from the perfused cat spleen by various ions and agents *J Physiol* (Lond) 1972 *221* 601—615

KOKETSU K Action of tetraethylammonium chloride on neuromuscular transmission in frogs *Amer J Physiol* 1958 *193* 213—218

STARKE K Influence of extracellular noradrenaline on the stimulation evoked secretion of noradrenaline from sympathetic nerves Evidence for an α receptor mediated feed back inhibition of noradrenaline release *Naunyn-Schmiedeberg's Arch exp Path Pharmak* 1972 *275* 11—23

STJARNE L Comparison of secretion of sympathetic neurotransmitter induced by nerve stimulation with that evoked by high potassium as trigger of dual alpha adrenoceptor mediated negative feedback control of noradrenaline secretion *Prostaglandins* 1973 a *3* 471—4 6

STJARNE L Michaelis Menten kinetics of secretion of sympathetic neurotransmitter as a function of external calcium *Effect of graded alpha adrenoceptor blockade Naunyn-Schmiedeberg's Arch exp Path Pharmak* 1973 b *278* 323—327

STJARNE L Role of alpha adrenoceptors in prostaglandin E mediated negative feedback control of the secretion of noradrenaline from the sympathetic nerves of isolated guinea pig vas deferens *Prostaglandins* 1973 c In press

THOENEN H W HAEFELY and H STAEHELIN Potentiation by tetraethylammonium of the response of the cat spleen to postganglionic sympathetic nerve stimulation *J Pharmacol exp Ther* 1967 *157* 532—540

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Muscle Spindles in a Flexion Reflex Elicited by Natural Stimulation

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Abstract

Appelberg B *Muscle spindles in a flexion reflex elicited by natural stimulation* Acta physiol scand 1973 *89* 145—153

In the decerebrate and unanesthetized cat spinalized at low thoracic level a flexion reflex in the peroneus tertius or the semitendinosus muscle was evoked by natural stimulation of the paw The behaviour of primary muscle spindle afferents from the muscle under phasic as well as static conditions was studied in the absence of as well as during a reflex contraction On the basis of the reactions of the spindles to these tests it could be concluded that during a flexion reflex evoked in a natural way static fusimotor neurones are active together with alpha motoneurones Dynamic fusimotor neurones on the other hand do not participate in the reflex

The mammalian muscle spindle is capable of measuring muscle length as well as velocity of length changes Its own motor supply also enables it to report to the central nervous system length as well as changes in length more or less vigorously From the reflex point of view the report is used to readjust extrafusal muscle length so as to release intrafusal tension

In retrospect it is easy to say that it seems utterly unlikely that such an ingenious sense organ should be put out of use every time its muscle contracts at least when the contraction leads to muscle shortening Merton (1953) as well as Granit (1955) foresaw this when they introduced the concepts of follow up length servo and α—γ linkage Since then a number of motor acts have indeed been shown to be caused by a simultaneous outflow in α and γ fibres (see Eklund Euler and Rutkowski (1964) and Sears (1964) for respiratory movements Taylor and Davey (1968) for jaw movements and Hagbarth and Vallbo 1968 and Vallbo (1970) for voluntary movements in man) In these studies the observation was made that the activity in muscle spindle afferents remains unchanged or even increases during a muscle contraction This necessarily implies an activity in static γ motoneurones together with the α activity

The duality of the fusimotor system originally demonstrated by Jansen and Matthews (1961 1962) with functionally independent routes for control of spindle

behaviour under static and dynamic conditions complicates the thinking of α–γ linkage How do the dynamic γ motoneurones enter the picture? Should we expect an α dynamic plus static γ linkage or an α static γ linkage only? Euler and Peretti (1966) concluded that static as well as dynamic γ motoneurones contributed to the spindle activation observed in the external intercostal muscle during breathing For the other motor acts studied any precise information regarding dynamic fusimotor activity is lacking Intuitively it is felt that a dynamic γ activation should accompany a movement whenever resistance against it is expected or possibly going to occur The auto regulation of the planned shortening (the load compensating reflex of C von Euler 1966) will then work at maximal efficiency Likewise it would seem favourable for a motor system to be able to set the dynamic sensitivity of its spindles if the system is going to maintain a steady contraction for some time irrespective of possible changes in load With optimal velocity sensitivity of the receptors the system may be able to counteract load rapidly and with minimal tendency of oscillation As a whole it thus seems likely that the more precise and critically controlled a movement or a steady contraction should be the more important a dynamic γ outflow concomitant with the α and static γ activity would be

The flexion reflex resulting from nociceptive stimulation represents a movement which is not precise and probably not critically controlled in the sense that in its protective role it mainly has to further some part of the body from a harmful stimulus Attempts have previously been made to investigate the nature of γ activation occurring during the flexion reflex Alnaes Jansen and Rudjord (1965) found evidence of dynamic γ activation in spinal animals upon repetetive electrical stimu of the ipsilateral saphenous nerve Bergmans and Grillner (1969) later demonstrated that the stimulation frequency used by the norwegian authors was unsuitable for reflex activation of static fusimotor neurones Upon electrical as well as natural stimulation evoking the flexion reflex Bergmans and Grillner found activation of two groups of γ fibres in the tenuissimus nerve These two groups were classified as static and dynamic with the aid of DOPA (*cf* Bergmans and Grillner 1967 and 1969) and on the basis of previous evidence concerning the influence of DOPA on primary and secondary muscle spindle endings (Bergmans and Grillner 1968 Grillner Hongo and Lundberg 1967)

The present experiments were designed to disclose how during a reflex muscle contraction caused by natural stimulation the spindles in the muscle are acted upon by their static and dynamic fusimotor neurones The experiments thus combined previously used techniques in that the reflex arc was intact and that the result of natural stimulation was observed in terms of extra and intrafusal effects

Methods

Experiments were performed on 17 cats In 11 of these a stabile flexion reflex occurred upon pinching the toes and the results presented below were essentially obtained in these 11 cats

Operation The operations which were performed with the animals anesthetized with halothane were initiated by transecting the spinal cord in the 12th or 13th thoracic segment. In a second laminectomy the dorsal roots S1 L7 and L6 were made available but all roots were usually left intact. Only in a few experiments where contralateral nerve stimulations were

planned the ventral roots S_1—L_5 were cut on the right side to prevent reflex contractions in the right leg The left hind limb was denervated except the nerve to the peroneus tertius (toe flexor) or the semitendinosus (knee flexor) muscles The cutaneous division of the superficial peroneal nerve was left intact The muscle under study was partially dissected free and its tendon cut Finally the cat was decerebrated and the anesthesia discontinued No recordings were made until at least one hour later when a good rigidity had developed in the fore limbs

Mounting The animals were mounted with rigid pins in the cristae iliacae and in the bodies of the 6th and 7th lumbar vertebrae as well as with a clamp in the spinous process of vertebra L_4 The left hind limb was supported partly by the skin forming the muscle pool partly by a steel pin through the proximal part of the tibia. The mechanical disturbance of the muscle by the manipulations with the paw was always checked and found to be negligible

Recording By recording first from intact thin natural divisions of the L_7 dorsal root the location of the muscle afferents in the root could be determined by listening to the loudspeaker while pulling the muscle and only a minor part of the root had to be cut for further dissection leaving the reflex connections essentially intact. Single primary muscle spindle afferents were then dissected and the reactions of these in terms of impulse frequency changes to the stimuli employed were used as an index of the fusimotor outflow to the muscle studied Usually the afferents were identified as being primary merely by watching and listening to their reactions to rapid stretches of the muscle The burst of impulses during muscle lengthening as well as the silence during the phase of release of stretch are characteristics which practically always separates primary from secondary spindle afferents Only very rarely a safe identification required a conduction velocity determination The electromyogram of the muscle was recorded with two Ringersoaked strands of cotton spread over the proximal and distal ends of the muscle

Stimulation The standard mechanical stimulus used to test spindle sensitivity was linear extension of the muscle usually a length change of 4 or 5 mm at a velocity of 5 or 10 mm/s The mechanical pulse used for stretching the muscle was produced by a stretching device consisting of a function generator producing a trapezoidal wave the rising and falling slopes of which could be independently varied in calibrated steps from 0 5 to 300 mm/s The duration of the wave could be set from 2 ms to 30 s The wave was fed into a power amplifier driving an electromognetic vibrator supplied with a differential transformer for deviation measurements The mechanical system had a movable mass of 10 g a mechanical resistance of 30 N s m^{-1} and a spring constant of 90 N m^{-1} The vibrator could produce a force of 20 N over the whole range of 10 mm To reduce the mechanical output impedance of the vibrator a negative feedback was applied from the deviation transducer to the power amplifier The total stiffness of the system amounted to 16 N m^{-1} To obtain sufficient damping of the system at different loads a derivating negative feedback was also employed The data mentioned thus give an unloaded frequency of 200 Hz and a damping ratio of 1 2 The deviation transducer had a range from DC to 300 Hz in the 3 dB range

To elicit the flexion reflex in the muscle under study the lateral toes were strongly pinched manually In attempts to selectively activate dynamic fusimotor neurones to the muscle studied the contralateral N peroneus communis or N tibialis were in 3 expts electrically stimulated strongly at frequencies around 30 Hz. These attempts became of value for interpreting the other results As the contralateral fusimotor effects as such were not studied specifically however nothing is known about the significance of such fusimotor reflexes

The dynamic index (see Crowe and Matthews 1964) given in the figures (D I) was calculated by subtracting the mean frequency in a 250 ms interval 0 5 s after the end of stretch from the mean frequency during the last 50 ms of stretch

All spindles recorded from (usually one in each experiment) were subjected to a large number of stretch tests (usually at least 25 during a period of several hours under control conditions as well as during reflex activation of the muscle Recordings representing typical and consistent observations made during these tests were photographed

Results

In the spinalized decerebrate and unanesthetized preparations used the primary endings studied under resting conditions as well as during stretch tests with the passive muscle usually revealed no definite signs of being under any kind of spontaneous fusimotor influence They had no or a low regular spontaneous activity with the muscle in the initial near to slack position Also with the muscle extended the spindle discharge was regular The burst during the phase of stretch was weak and remain

of dynamic bursts seen in deefferented spindles. Upon release of stretch a period of silence was seen. Only in one exceptional case a rather high frequency irregular discharge in combination with a lack of pause upon release of stretch indicated a rather strong spontaneous static fusimotor influence (*cf.* the spindle in Fig. 1). Admittedly, however, as the spindles were *not* tested again after deefferentation, a weak spontaneous fusimotor outflow (dynamic and/or static) also to other spindles may have occurred.

Upon pinching the foot (tactile stimuli were not effective) the muscle under study in *2* of the 11 preparations contracted in what might be called an alpha contraction. In these instances the primary endings studied were silenced *during* the contraction and a possible simultaneous fusimotor outflow was at least too weak to match the muscle shortening. These experiments were regarded as *failures* as they were in minority and seemed to result from cats in less good condition. They do, *however, demonstrate that the flexion reflex may occur without the support of activated spindles*, i.e. as a pure alpha contraction.

Much more commonly, however, i.e. in 9 out of the 11 preparations where a reflex occurred, the reflex contractions took place during signs of a *simultaneous* skeletomotor and fusimotor outflow. Results from an experiment illustrating this are shown in Fig. 1. The primary spindle afferent used in this experiment is the exceptional one already mentioned. It thus exhibited signs of a rather strong static background fusimotor activation already in the resting state (Fig. 1A). Its dynamic sensitivity was low, the burst during the extension phase being practically hidden by the irregular static discharge. During mechanical stimulation of the foot the semitendinosus muscle contracted but instead of being unloaded the spindle further increased its *frequency* and its discharge became even more irregular (Fig. 1B). It

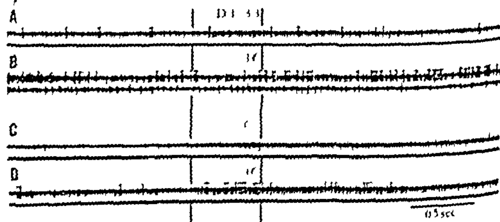


Fig. 1. Primary muscle spindle afferent from the [illegible] muscle. Muscle [illegible] 3 mm at 10 mm/s between vertical lines. [illegible] each [illegible] cord. A, control [illegible] B, [illegible] flex[illegible] paw [illegible] acceleration injection of Flax[illegible] (0.40 mg/kg. D, [illegible] but no muscle activity. Note decrease of dynamic [illegible] paw stimulation. Note also the presence of an additional spike in the [illegible] frequency and is probably [illegible]

should be particularly pointed out that any sign of a simultaneous increase of the dynamic sensitivity is lacking The dynamic burst is completely hidden in the vigorous static discharge and the dynamic index is lowered as should be expected during a static fusimotor activation (Crowe and Matthews 1964)

To ascertain that the increased static discharge was not due merely to extrafusal mechanical stimulation of the spindle the same tests were repeated after blocking the extrafusal endplates with Flaxedil (Fig 1 C and D) Under control conditions the spindle was now somewhat less active (C) presumably due to some effect of the Flaxedil also on intrafusal motor endings A strong intrafusal activation of a purely static type was however caused by foot stimulation in spite of the fact that the muscle it elf was now not active (D) Results to the same effect were obtained in all 6 experiments where Flaxedil was used (of these 6 expts 5 were on the peroneus tertius 1 on the semitendino us muscle)

The maintenance or even acceleration of the spindle discharge during the contraction of the peroneus tertius (7 expts) or the semitendinosus (2 expts) muscle in a flexion reflex clearly indicates a parallell activation of alpha and static fusimotor neurones in this reflex On the other hand the attempts to test spindle dynamic sensitivity by the application of linear extension failed to reveal any signs of a simultaneous dynamic fusimotor outflow

Before reaching the conclusion that the dynamic sensitivity of the spindles is not increa ed during the flexion reflex studied it is however necessary to try to answer the following questions 1 may the usual signs of increased dynamic sensitivity be obscured due to the fact that the spindle is simultaneously statically activated? 2 may an increased dynamic sensitivity be impossible to see when the surrounding muscle is in contraction and can the dynamic fusimotor effects be hidden also when the extrafusal muscle is curarized due to rather similar thresholds of extra and intrafusal endplates to Flaxedil (*cf* Emonet Denand and Houk 1968)? 3 could it be that by pure misfortune the spindle endings studied were of a type with only a very weak dynamic fusimotor innervation?

Ad question 1 Lennerstrand (1968) studied interaction between two fusimotor fibres converging *to the same spindle When pairs of a static and a dynamic fusi* motor fibre were used the velocity sensitivity of the spindle remained es entially the same as it was when the dynamic fibre was stimulated alone Any masking of the velocity sensitivity to increments in muscle length due to a simultaneous static activation of the spindle was thus not seen Appelberg and Jeneskog (1972) studied changes in spindle sensitivity caused by the simultaneous activation of descending systems influencing static and dynamic fusimotor neurones The change in dynamic en itivity cau ed by the central stimulus was seen equally well before and after a lesion interrupting the path to the static neurones (*cf* their Fig 5 and 6) It thus seems conclusively demonstrated that activity in dynamic fusimotor fibres will be revealed by the stretch test even when activity in static fibres is simultaneously going on

Ad question 2 Accepting the possibility that extra and intrafusal motor end plates (Barker Emonet Denand Laporte Proske and Stacey (1970) sl

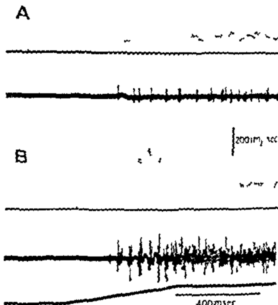


Fig 2 Primary muscle spindle afferent from the long digital flexor muscle Instantaneous frequency recording with zero frequency indicated by black line Electromyographic recording below in each record A muscle extended 5 mm at about 10 mm/s under control conditions B same during repetitive electrical stimulation in a mesencephalic area influencing dynamic fusimotor neurones Records retouched

endings to be trail endings thus dynamic endings are apt to be plate endings) are approximately simultaneously blocked by curare it is necessary to show that dynamic fusimotor effects may be seen al o in a muscle under contraction In Fig 2 which presents recordings from unpublished work by the present author this is clearly een to be the case Recording A was obtained in the lightly anesthetized cat with ntact dorsal and ventral roots When the long digital flexor muscle was stretched weak stretch reflex developed In B a mesencephalic area influencing dynamic usimotor neurones was stimulated repetetively The passive extension of the muscle now caused a considerably stronger stretch reflex with a vigorous contraction of the mu cle Probably due to the high dynamic sensitivity of the spindles the contraction became oscillatory during the phase of extension In spite of the trong oscillatory contraction the marked increa e of spindle dynamic ensitivity i clearly seen There seems to be no rea on to suspect therefore that in any muscle a high dynamic spindle sensitivity hould not be een al o in the contracting muscle

Another reply to *que tion 2* may be obtained by referring to the work of Emonet Denand and Laporte (1966) where intrafusal β plates were shown in fact to be less ensitive to curare than extrafusal motor endings This information was utilized in one of the three experiments of the present series where a contralateral nerve stimulus caused an increased dynamic ensitivity of the spindle under study It was *quite evident* in that experiment that a change of dynamic sensitivity of a spindle could be obtained also when the extrafusal muscle was blocked by Flaxedil Also in this *quite crucial situation* however the flexion reflex was established as an increased static fusimotor activity only

This experiment *together* with two others employing contralateral nerve stimulation gives a reply also to *question 3* above It seemed necessary to show that a spindle behaving as all the others studied during the flexion reflex could from some

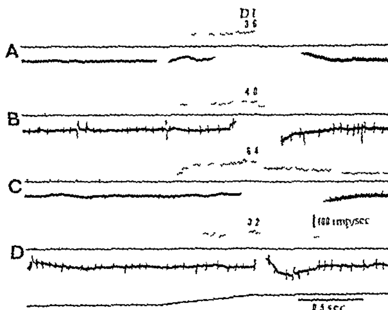


Fig 3 Primary muscle spindle afferent from the peroneus tertius muscle Instantaneous frequency recording with zero frequency indicated by black line Electromyographic recording below in each record (interupted by drifting off screen due to inadequate time constant) A muscle extended 4 mm at 5 mm/s under control conditions B same but flexion reflex evoked in muscle by pinching the paw C repetetive electrical stimulation of contralateral common peroneal nerve causing activation of dynamic fusimotor neurones D pinch of paw and contralateral nerve stimulation combined Flexion reflex evoked but effect on dynamic fusimotor neurones blocked Dots in frequency recordings retouched

other input be influenced also toward an increased dynamic sensitivity Fig 3 is from such an experiment In A is shown the response of the spindle to stretch of the passive muscle B was obtained while pinching the foot In this case the static fusimotor outflow is exactly balancing the shortening of the contracting muscle and the spindle behaves as in the control except for being somewhat more irregular in its firing No signs of dynamic fusimotor activation is seen In C a clearcut dynamic activation is brought about by repetetive stimulation of the contralateral common peroneal nerve Another interesting observation is demonstrated in Fig 3 D where the two stimuli i e the mechanical paw stimulus and the electrical nerve stimulus were combined A flexion reflex with static fusimotor activation is evoked much the same as in B but the effect of the nerve stimulus is actually abolished

Discussion

These results should be seen in relation to those of Alnaes *et al* (1965) and to those of Bergmans and Grillner (1967 1968 1969) First these two groups of In the experiments presented clear evidence for a parallel activation of skeletomotor and static fusimotor fibres in the flexion reflex was obtained On the other hand the experiments failed to disclose any signs of a simultaneous increase in the dynamic fusimotor outflow

workers both reported a predominance of activity in dynamic fusimotor neurones in the spinalized cat under resting conditions It is quite clear that in the present experiments where spindle sensitivity before and after deefferentation was not compared a weak spontaneous dynamic fusimotor outflow may have occurred Secondly Alnaes *et al* found that electrical stimulation of the saphenous nerve causing a flexion reflex in the anterior tibial muscle only caused an increased dynamic fusimotor drive Bergmans and Grillner (1969) later found at other stimulating frequencies activation of dynamic as well as static neurones to the tenuissimus muscle with sural nerve stimulation Bergmans and Grillner also reported reflex activation of both types of γ units to the tenuissimus muscle upon adequate stimulation of the paw

That *the reflex effects of electrical stimulation may be different from those ob*tained with natural stimulation is not surprising Groups of nerve fibres not participating in the natural reflex may well be activated by the electrical stimulus On the other hand Bergmans and Grillners observation of dynamic activation also by an adequate stimulus of the same kind as the one used in the present experiments may seem remarkable and contradictory A simple explanation could be however that small changes in fusimotor outflow, though easy to observe when recording directly from the motor axons may be of minor or no significance for spindle sensitivity

The remarkable depression of a contralateral inflow to dynamic fusimotor neurones during a simultaneously elicited flexion reflex (Fig 3 D) also merits discussion This is not the first evidence obtained for a certain degree of reciprocity between actions on static and dynamic fusimotor neurones Bergmans and Grillner (1967 and 1969) observed a reciprocity in the action of DOPA on the spontaneous activity of two groups of fusimotor neurones classified as being static and dynamic They suggested as a reasonable explanation of this an inhibitory interneuronal chain from the pathway to dynamic fusimotor neurones to the one to static neurones the latter being released from a tonic inhibitory influence by the action of DOPA Such an inhibitory action from the dynamic to the static path could well exist and be put into operation by the electrically evoked flexion reflex which seems to favour dynamic activation in the spinal animal not treated with DOPA Its tonic effect on the path to static neurones must be mild however as natural stimulation easily breaks through to static fusimotor neurones

A similar line of thinking may however be applied to explain the present findings Flexion reflex afferent activity mainly directed to alpha and static fusimotor neurones may also be thought to inhibit ongoing activity in a contralateral pathway to dynamic fusimotor neurones Such an explanation implies that while in the flexion reflex alpha and static fusimotor neurones are active it could for some yet unknown reason be beneficial that the dynamic neurones are kept at a low level of activity A discussion of such an interpretation of the present results is however better postponed until much more knowledge has accumulated concerning the functional role of the dynamic fusimotor neurones

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References

ALNAES E J K S JANSEN and T RUDJORD Fusimotor activity in the spinal cat *Acta physiol scand* 1965 *63* 197—212

APPELBERG B and T JENESKOG Mesencephalic fusimotor control *Exp Brain Res* 1972 *15* 97—112

BARKER, D F EMONET DENAND Y LAPORTE U PROSKE and M STACEY Identification des terminaisons motrices des fibres fusimotrices statiquez chez le chat. *C R Acad Sci* (Paris) Ser D 1970 *271* 1203—1206

BERGMANS J and S GRILLNER, Reflex activation and regulation of spontaneous activity in static and dynamic motoneurones *Brain Res* 1967 *5* 114—117

BERGMANS J and S GRILLNER Changes in dynamic sensitivity of primary endings of muscle spindle afferents induced by DOPA *Acta physiol scand* 1968 *74* 629—636

BERGMANS J and S GRILLNER Reciprocal control of spontaneous activity and reflex effects in static and dynamic flexor γ motoneurones revealed by an injection of DOPA *Acta physiol scand* 1969 *77* 106—124

CROWE A and P B C MATTHEWS The effects of stimulation of static and dynamci fusimotor fibres on the response to stretching of the primary endings of muscle spindles *J Physiol* (Lond) 1964 *174* 109—131

EKLUND G C v EULER and S RUTKOWSKI Spontaneous and reflex activity of intercostal gamma motoneurones *J Physiol* (Lond) 1964 *171* 139—163

EMONET DENAND F and J HOUK Etude comparative de la curarisation des synapses neuromusculaires des fibres fusimotrices γ dynamiques et statiques chez le chat *J Physiol* (Paris) 1968 *60* 367—372

EMONET DENAND F and Y LAPORTE Persistance de l action dynamique des fibres motrices β sur les terminaisons primaries de fuseaux après curarisation des jonctions neuromusculaires extrafusales *C R Acad Sci* (Paris) Ser D 1966 *263* 1405—1407

EULER C v The control of respiratory movement In *Breathlessness* (Eds J B L Howell and E J M Campbell) Oxford Blackwell Sci Publ 1966 pp 19—32

EULER, C v and G PERETTI Dynamic and static contribution to the rhytmic γ activation of primary and secondary spindle endings in external intercostal muscles *J Physiol* (Lond) 1966 *187* 501—516

GRANIT R *Receptors and Sensory Perception* New Haven Yale University Press 1955

GRILLNER S T HONGO and A LUNDBERG The effect of DOPA on the spinal cord 7 Reflex activation of static γ motoneurones from the flexor reflex afferents *Acta physiol scand* 1967 *70* 403—411

HAGBARTH K E and A B VALLBO Discharge characteristics of human muscle afferents during muscle stretch and contraction *Exp Neurol* 1968 *22* 674—694

JANSEN J K S and P B C MATTHEWS The dynamic response to slow stretch of muscle spindles in the decerebrate cat *J Physiol* (Lond) 1961 *159* 20P—22P

JANSEN J K S and P B C MATTHEWS The central control of the dynamic response of muscle spindle receptors *J Physiol* (Lond) 1962 *161* 357—378

LENNERSTRAND G Position and velocity sensitivity of muscle spindles in the cat IV Interaction between two fusimotor fibres converging on the same spindle ending *Acta physiol scand* 1968 *74* 257—273

MERTON P A Speculations on the servo-control of movement *The Spinal Cord* Ciba Symposium. Churchill London 1953 247—260

SEARS T A. Efferent discharges in alpha and fusimotor fibres of the intercostal nerves of the cat *J Physiol* (Lond) 1964 *174* 295—315

SEVERIN F V G N ORLOVSKIJ and M L SHIK Work of the muscle receptors during controlled locomotion *Biophysic* 196 *1*? 5?5—586

TAYLOR A and M R DAVEY Behaviour of jaw muscle stretch receptors during active and passive movements in the cat *Nature* Lond) 1968 *220* 301—302

VALLBO A B Slowly adapting muscle receptors in man *Acta physiol scand* 1970 *78* 315—333

Acta physiol scand 1973 89 154—168

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Colonic Motility in the Cat

IV Peripheral Pathways Mediating the Effects Induced by Hypothalamic and Mesencephalic Stimulation

By

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Abstract

ROSTAD H *Colonic motility in the cat IV Peripheral pathways mediating the effects induced by hypothalamic and mesencephalic stimulation* Acta physiol scand 1973 *89* 154—168

The peripheral pathways by which the hypothalamus and mesencephalon influence the motility of the colon have been studied by combining brain stimulation and peripheral nerve sectioning and by use of autonomic blocking agents *Excitatory* colonic effects induced from the hypothalamic sympatho inhibitory area with concomitant blood pressure falls were found to be mediated through the sympathetic lumbar colonic nerves whereas excitatory responses with associated blood pressure rises were conveyed through the latter as well as through the parasympathetic pelvic nerves On the other hand the augmentatory colonic effects usually associated with blood pressure rise evoked from the mesencephalon were conveyed through the lumbar colonic nerves only The responses through the lumbar colonic and pelvic nerves were blocked by guanethidine and atropine respectively The augmentatory colonic responses from the hypothalamic sympatho inhibitory area were blocked by the beta adrenergic blocking agent propranolol whereas the effects from the other responsive hypothalamic area transmitted through the lumbar colonic nerves were blocked by alpha blocker (phenoxybenzamine) but not by propranolol This finding support the presence of alpha excitatory receptors in the colonic wall

Inhibition of colonic motility induced by hypothalamic and mesencephalic stimulation were found to be mediated through the lumbar colonic as well as through the splanchnic nerves The effects were blocked by guanethidine and those from the responsive hypothalamic zones were also blocked by propranolol

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The peripheral pathways conveying the centrally induced effects on colonic motility are not known although results of transection of the spinal cord have stressed the importance of the sacral outflow for the hypothalamic control (Ingersoll and Jones 1946) Apart from this almost no information about the peripheral pathways is available Other visceral activities have been studied by using a combination of central stimulation and sectioning of the peripheral nerves This requires knowledge of the peripheral pathways as well as the effects of stimulating and cutting these nerves under similar conditions in the same species and with the same recording

technique as that used during brain stimulation Such knowledge with respect to the colon in the cat was gained in a previous study (Rostad 1973 b) in which 3 known excitatory pathways (vagal pelvic and lumbar colonic) and 2 inhibitory pathways (splanchnic and lumbar colonic) were analyzed

The validity of the conclusions drawn from the use of such combined techniques for studying the stomach has recently been questioned by Jansson Lisander and Martinson (1969) who claimed that some degree of background activity of the vagal excitatory fibres is a prerequisite for revealing changes in activity of sympathetic neurons to the stomach Corresponding objections might also be applied to studies of the colon Therefore, the effects of nerve sectioning were studied using stimulation of the cerebral structures and the peripheral ends of the cut parasympathetic colonic nerves

The *purpose* of the present work was to trace the efferent peripheral pathways mediating the colonic excitatory and inhibitory effects obtained by stimulation of various hypothalamic and mesencephalic areas which have been described in a preceding report (Rostad 1973 c) and which are listed in Table I The motility of the colon was recorded with a sensitive extraluminal strain gage transducer technique (Rostad 1973 a) and the effects of brain stimulation have been studied after section of the various extrinsic colonic nerves in question

It is a well established fact that both alpha and beta inhibitory adrenergic receptors are present in the intestine (Lee 1970) Further there is a possible admixture of cholinergic fibres in the sympathetic outflow to the colon (Rostad 1973 b) Therefore the effects of alpha and beta blockers as well as agents blocking adrenergic or cholinergic transmission have been studied

Material and methods

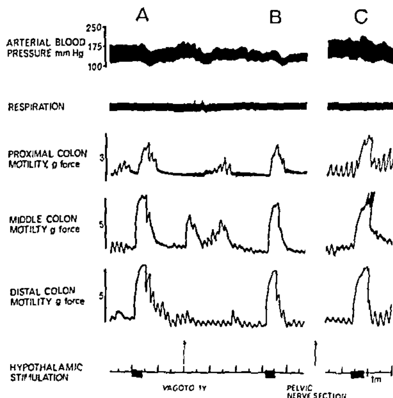
The results are based on data gained from 73 cats mostly male weighing from [illegible] to 4 8 kg Some of the animals were also used in the study of other cerebral structures which will be dealt with in a subsequent communication (Rostad 1973 d) The preoperative regime was as described in a previous report (Rostad 1973 a) Most animals were anesthetized with penthobarbital sodium (Nembutal® Abbott) 30 mg/kg b w i p and some with chloralose 30—40 mg/kg b w i v after induction with ether The neuromuscular blocking agent pancuronium bromide (Pavulon® Organon) was administered i v in amounts of 0 1—0 3 mg/kg b w

The *operative procedures* and *recording techniques* were as described previously (Rostad 1973 a) In brief the motility of the proximal the middle and the lower parts of the bowel was recorded as well as the arterial blood pressure and the respiratory movements The catecholamines from the adrenal glands were excluded from the c ulat on b a eful ligation of the vessels of both glands Adrenocortical substitution as s cured by i inje tion of hydrocortison (Solu Cortef® Upjohn) 5—10 mg/kg b w

Other drugs were administered i atropine (Atropin® 0 1 NAF 0 5—1 0 mg/kg b w guanethidine (Ismelin® Ciba) 4 mg/kg b w hexamethonium bromide 1 mg/kg b phenoxybenzamine 2—4 mg/kg b w and propranolol (Inderal® I C I) 0 5—1 0 mg kg b

Electrical stimulation of the brain the *histological* identification of the electrode placements *e t ins c nerv se tion and stimulation* were performed as described in preceding reports (Rostad 1973 b c)

When a consistent centrally induced colonic effect had been secured the extrinsic nerves in question were cut and the central stimulation repeated Because of the hypermotility following section of the sympathetic nerves and because of the exten surgical interference associated with pelvic nerve section the central stimulation ould in uch experiment only be repeated after a delay of 15—30 min For these reasons the e t insic n rves n qu tion were divided in 8 expts during the initial operation



1 Cat 4 3 kg Excitatory colonic responses on stimulation of the sympatho-inhibitory area the anterior hypothalamus before (*A*) and after bilateral cervical vagotomy (*B*) and after additional bilateral pelvic nerve section (*C*) Note the unaltered colonic effects of hypothalamic stimulation after the nerve sections Stimulus parameters 100 Hz 1 ms 3 V

For peripheral nerve stimulation square wave pulses at frequencies of 10—20 Hz of 2 ms duration and at intensities of 1—15 V were commonly used

Results

The effects of hypothalamic and mesencephalic stimulation on colonic motility following section of various extrinsic nerves are summarized in Table I which also gives the number of animals used in each type of experiment

Excitatory responses

Following bilateral cervical *vagotomy* the increased colonic activity produced from all the excitatory points in the hypothalamus and mesencephalon remained unaltered (Fig 1 A B) This was also the case after selective bilateral *pelvic* nerve section The responses still persisted after section of both the vagal and pelvic nerves and the amplitude of the contraction waves was usually not reduced (Fig 1 C) To be sure that section of the pelvic nerves was complete a tight ligature was placed around the sigmoid colon in some experiments

Similarly bilateral section of the *splanchnic nerves alone* (Fig 2 A B) or in

TABLE I Effects on colonic motility of hypothalamic and mesencephalic stimulation after bilateral section of various extrinsic nerves as indicated in the left column Figures within parenthes The number of animals stimulated Figures outside parenthesis The number of these animals in which the responses indicated on the top were obtained after nerve section Framed figures No response

		Area stimulated								
		Excitatory responses						Inhibitory responses		
Nerves sectioned	—	Sympatho-inhibitory area	Ventral anterior hypothal	*Lateral* hypothal	*NIIvm* (dorsal part)	*Posterior* hypothal	Mesencephalon	Medial forebrain bundle	NIIvm (ventral part)	Mesencephalon
Vagal	(3)	11 (1)	14 (15)	5 (7)	1 (1)	? (3)	5 (6)	3 (4)		3 (4)
Pelvic	(18)	9 (13)	11 (1)	5 (5)	3 (3)	1 (1)	3 (3)	2 (?)	1 (1)	2 (3)
Vagal and pelvic	(33)	?0 (?5)	7 (8)	19 (19)	14 (15)	11 (14)	, (8)	14 (16)	2 (4)	5 (8)
Splanchnic	(5)	4 (5)	? (5)	4 (?)	4 (4)	3 (5)		1 (3)	1 (2)	
Lumbar colonic	(7)	0 (5)	4 (?)	4 (?)	3 (4)	1 (?)		? (4)	0 (1)	
Splanchnic and lumbar colonic	(14)	0 (9)	8 (?)	9 (9)	7 (8)	, (7)	0 (5)	0 (6)	0 (4)	0 (4)
Vagal pelvic and splanchnic	(9)	4 (?)	5 (5)	6 (?)	5 (6)	4 (5)	? (3)	(3)	? ( )	? ( )
Pelvic and lumbar colonic	(5)	0 (?)	0 (?)	0 (3)	0 ( )	0 (3)	0 ( )			1 (1)
Vagal pelvic and lumbar colonic	(8)	0 (4)	0 (4)	0 (5)	0 (3)	0 (4)	0 (?)	4 (6)	2 (3)	1 (2)
All nerves cut	(3)	0 (?)	0 (1)	0 (1)	0 (?)	0 (?)	0 (1)	0 (1)	0 (1)	0 (1)

combination with both parasympathetic nerves did not seem to reduce the centrally induced colonic excitatory responses

An important pathway for the excitatory effects appears to be the sympathetic lumbar colonic nerves which contribute to the effects evoked from all hypothalamic and mesencephalic areas

(a) Stimulation of the hypothalamic sympatho inhibitory area (Folkow Johansson and Öberg 1959) produced colonic contractions and a concomitant fall in blood pressure after section of the parasympathetic vagal and pelvic nerves

Selective section of the lumbar colonic nerves or section of both the splanchnic and lumbar colonic nerves abolished the responses from the sympatho-inhibitory area (Fig 3 A B)

(b) The effects produced by stimulation of the other hypothalamic areas where the increase in colonic motility was associated with a rise in blood pressure and increased pulse rate remained almost unchanged after section of the splanchnic or the lumbar colonic nerves or when both nerves were sectioned together (Table I Fig 2 C and 3 C) This was also the case after additional section of the vagal nerves Thus from these hypothalamic areas the effects were mediated through both the pelvic and the lumbar colonic nerves

(c) Stimulation of mesencephalic areas after section of the splanchnic and lumbar colonic nerves did not produce excitatory responses (Table I Fig 4) indicating that the *colonic effects do not pass through the parasympathetic nerves*

After section of the pelvic and lumbar colonic nerves the excitatory colonic effects from all hypothalamic and mesencephalic areas were abolished indicating neither the vagal nor the splanchnic nerves contain excitatory pathways that be detected with the method employed Thus among the sympathetic nerves only the lumbar colonic was *found to mediate excitatory colonic responses* Similar results were obtained in experiments in which only the splanchnic or lumbar colonic nerves were left intact and the *cerebral structures as well as the peripheral ends* of the vagal or pelvic nerves were stimulated simultaneously

The pelvic nerves appear to mediate excitatory colonic effects from all responsive hypothalamic zones with exception of the sympatho inhibitory area The vagal nerves on the other hand do not seem to play any important role *for the* hypothalamic and mesencephalic excitatory *influence on the colon*

Inhibitory responses

Inhibitory colonic responses have been obtained from the ventral part of the hypothalamus mainly corresponding to the course of the medial forebrain bundle the ventral part of the ventromedial nucleus and the mesencephalic central tegmental tract and medial lemniscus (Rostad 1973 c)

As seen from Table I these inhibitory colonic effects persisted after bilateral section of either the *vagal* and *pelvic* nerves as well as after section of both The inhibitory responses were usually easier to elicit when abundant *colonic motility* was induced by just suprathreshold stimulation of the peripheral end of the cut vagal and pelvic nerves (Fig 5)

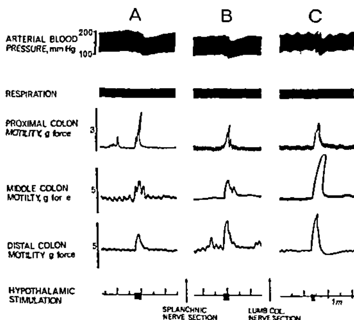
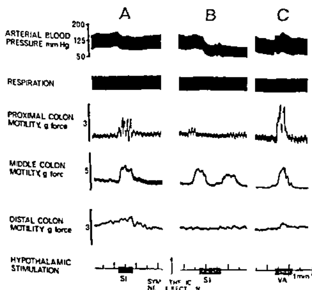


Fig 2 Cat 3 9 kg Excitatory colonic respon es on stimulation of the lateral h pothalamus before (*A*) and after section of the splanchnic nerves (*B*) and then of lumbar colonic nerves (*C*) The effects were still easily produc d after the nerve sections Stimulus parameters 100 Hz 1 ms 3 V



F g 3 Cat 2 3 kg (*A*) Colon c responses from the h pothalam c sympatho-inhi bitory area (SI) are abolished by section of the splanchnic and lumbar colonic nerves (*B*) whereas the effects from the ventral anterior hypothalamus (VA) are still elicit able (*C*) Stimulus parameters 100 Hz 1 ms 3 V

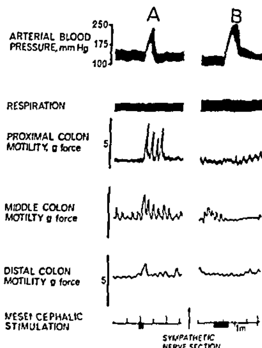


Fig 4 Cat 4 0 kg Excitatory colonic responses on mesencephalic stimulation (*A*) are abolished by section of the *splanchnic and lumbar colonic nerves (B)* Stimulus parameters 80 Hz 1 ms 4 V

Section of the *splanchnic* nerves alone did not abolish the inhibitory effects of hypothalamic stimulation Also after combined section of the splanchnic and parasympathetic nerves colonic inhibition from hypothalamic and mesencephalic structures was still obtained (Table I Fig 6) Thus inhibition appears to be mediated through the *lumbar colonic* nerves After selective section of the latter the inhibitory responses from the medial forebrain bundle remained unchanged whereas effects from the ventromedial hypothalamic nucleus were not elicitable (one animal) However experiments with combined section of the lumbar colonic and parasympathetic nerves demonstrated that inhibitory colonic responses could still be produced from the hypothalamic as well as from the mesencephalic areas when the splanchnic nerves only were left intact (Table I) These effects were usually weak and most conspicuous in the proximal part of the colon

Inhibitory responses induced by hypothalamic or mesencephalic stimulation were not obtained when the splanchnic as well as the lumbar colonic nerves had been divided Thus the parasympathetic nerves do not appear to mediate inhibitory effects to the colon

After section of all extrinsic nerves the excitatory as well as inhibitory responses from hypothalamic and mesencephalic areas were abolished thus providing evidence for a nervous pathway

Influence of blocking agents

Brain stimulation was not undertaken until at least 30 min had elapsed after administration of the drugs The effects are summarized in Table II

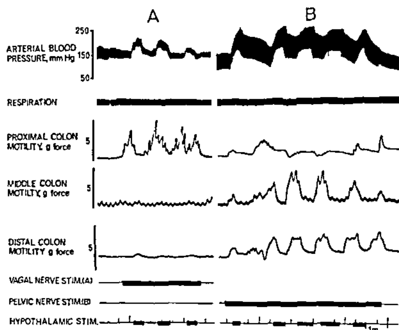


Fig 5 Cat 4 0 kg Vagal and pelvic nerves divided and colonic motility produced by continuous stimulation of the peripheral ends of the vagal and pelvic nerves
A Vagally induced proximal colonic motility is inhibited by stimulation in the region of the medial forebrain bundle in the medial hypothalamus (80 Hz 1 ms 3 V) Vagal nerve stimulation 8 Hz 2 ms 2 V
B Colonic motility induced by pelvic nerve stimulation (8 Hz 2 ms 1 V) most conspicuous in the lower part is inhibited by stimulation of the medial forebrain bundle in the posterior hypothalamus

All extrinsic nerves intact Atropine in doses of 0 5—1 0 mg/kg b w did not block either the excitatory or the inhibitory colonic effects produced by hypothalamic stimulation The response from the posterior hypothalamus was not elicitable after atropine but since there was only one animal in this group judgement concerning this effect must be reserved

Guanethidine which blocks adrenergic transmission postsynaptically in amounts of 4 mg/kg b w abolished the augmentatory colonic effects from the sympatho-inhibitory area as well as the inhibitory effects from the ventromedial nucleus and the region of the medial forebrain bundle indicating an adrenergic transmission in the pathways from these areas The excitatory colonic responses from the other responsive hypothalamic areas remained uninfluenced However atropine and guanethidine in combination blocked the colonic effects from all responsive hypothalamic areas indicating both an adrenergic and a cholinergic transmission Likewise all effects were abolished by the ganglion blocking agent hexamethonium 1 mg/kg b w

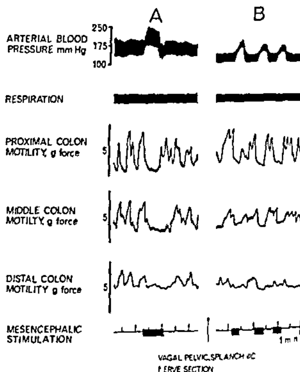


Fig 6 Cat 2 6 kg Inhibitory colonic responses on stimulation of the mesencephalic central tegmental tract (*A*) are uninfluenced by bilateral section of the vagal the pelvic and the splanchnic nerves (*B*) Stimulus parameters 80 Hz 1 ms 3 V

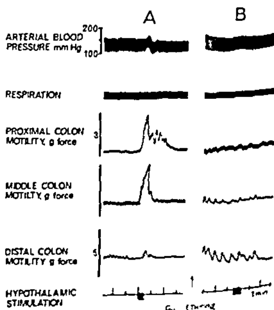


Fig 7 Cat 2.2 kg Vagal and pelvic nerves divided Excitatory colonic responses on stimulation of the ventromedial hypothalamic nucleus (*A*) are blocked by guanethidine 4 mg/kg b w (*B*) Stimulus parameters 30 Hz 1 ms 3 V

Fig 8 Cat 2 9 kg Vagal and pelvic nerves divided Influence of propranolol and phenoxybenzamine on the colonic effects of hypothalamic stimulation (*A*) Responses from the sympatho-inhibitory area (SI) and from the ventral part of the anterior hypothalamus (VA) before administration of the drugs The former is blocked by propranolol 0 5 mg/kg b w (*B*) while the latter remains almost unaltered (*C*) but is abolished by phenoxybenzamine 4 mg/kg b w (*D*) Stimulus parameters 80 Hz 1 ms 3

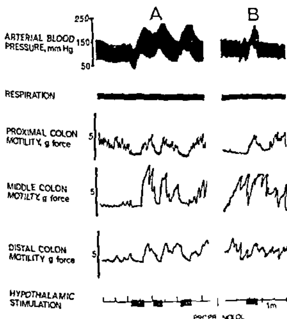


Fig 9 Cat 3 4 kg Vagal and pelvic nerves divided Inhibitory colonic responses from the region of the medial forebrain bundle (*A*) are blocked by propranolol 1 mg/kg b w (*B*) Stimulus parameters 100 Hz 1 ms 4

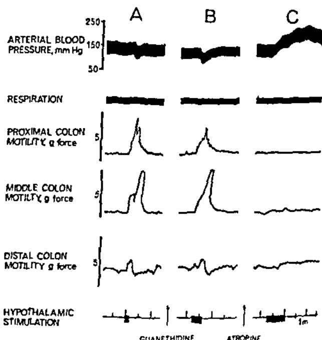


Fig 10 Cat 3 kg Splanchnic and lumbar colonic nerves divided Colonic responses from the ventromedial hypothalamic nucleus (*A*) are almost uninfluenced by *guanethidine* 4 mg/kg b w (*B*) but are completely blocked by atropine 0 5 mg/kg b w (*C*) Stimulus parameters 100 Hz 1 ms 3 V (The last stimulus 10 V)

Vagal and pelvic nerves divided After atropine all colonic responses excitatory well as inhibitory from hypothalamic and mesencephalic structures were still citable (Table II)

Guanethidine on the other hand completely blocked all colonic effects from both the hypothalamus and the mesencephalon as examplified in Fig 7 Thus excitatory as well as inhibitory colonic effects seem to be mediated through adrenergic fibres within the sympathetic nerves However as the splanchnic nerves do not seem to convey excitatory colonic effects in the parasympathectomized colon these are mediated through the lumbar colonic nerves

The beta adrenergic blocking agent propranolol in amounts of 0 5—1 0 mg/kg b w blocked the augmentatory colonic effects from the sympatho inhibitory area (Fig 8 A B) as well as the inhibitory effects from the medial forebrain bundle and the ventral part of the ventromedial nucleus (Fig 9) However the excitatory colonic responses from the other hypothalamic areas and the mesencephalon were almost unaffected (Table II Fig 8 C)

The alpha adrenergic blocking agent phenoxybenzamine in doses of 2—4 mg/kg b w completely blocked the excitatory colonic effects from all responsive hypothalamic and mesencephalic areas (Fig 8 D)

Thus beta adrenergic receptors seem to be involved in the inhibition of colonic motility as well as in the augmentatory effect from the sympatho-inhibitory area while alpha adrenergic receptors are involved in the transmission of excitatory colonic effects from other responsive hypothalamic and mesencephalic structures

BLE II Effects on colonic motility of hypothalamic and mesencephalic stimulation after ad ministration of pharmacological blocking agents as indicated to the left Figures within parenthesis The number of an mals stimulated Figures outside parenthesis The number of these animals in which the responses indicated on the top were still obtained after administration of the drug Framed figures No response

			Area stimul ted								
			Excitatory r sp ses						Inh b tory respo ses		
	Nerv s ti d		Symp tho-inhibitory re	Ventral nterior hypothal	L teral hypothal	NIIvm (dors l p rt)	P terior hypothal	Mesen cephalon	M d al for bra n bundle	NII m (v ntral part)	Me n cephal n
All int trinsic nerves	At p	(3)	1 (2)	3 (3)	3 (3)	3 (3)	0 (1)		2 (2)	1 (1)	
	Guan th d	(3)	0 (3)	3 (3)	3 (3)	1 (2)	1 (3)		0 (3)	0 (3)	
	Atrop ne + Gu eth dine	(1)	0 (1)	0 (1)	0 (1)	0 (1)	0 (1)		0 (1)	0 (1)	
	H xam th m	( )	0 ( )	0 (2)	0 (2)	0 (?)	0 (2)		0 (2)	0 (2)	
V gal and pd nerv s d d	Atr pine	(13)	3 (6)	5 (7)	8 (8)	7 (8)	(4)	? (?)	5 (5)	3 (3)	
	Gu n th d e	(1)	0 (5)	0 (5)	0 (7)	0 (4)	0 (5)	0 (3)	0 (5)	0 (4)	0 (3)
	Prop n lol	(8)	0 (5)	6 (6)	6 (6)	4 (4)	? ( )	( )	0 (3)	0 (?)	
	Phenoxy b m ne	(3)	0 ( )	0 ( )	0 (?)	0 (2)	0 (2)	0 ( )			
Splanchni and lumb of nerves d pd d	Gu neth d ne	(5)		> (3)	4 (5)	4 (4)	? (3)				
	At pin	(2)		0 (1)	0 (2)	0 ( )	0 (1)				

Splanchnic and lumbar colonic nerves divided The colonic effects from hypothalamic stimulation mediated through the pelvic nerves were unaffected by guanethidine but were blocked by atropine indicating a cholinergic transmission (Fig 10)

Discussion

The present experiments demonstrate a dual peripheral pathway for the excitatory colonic effects induced by stimulation of hypothalamic structures except for the sympatho inhibitory area Section of one of the two pathways usually did not reduce the response indicating a strong colonic effect through each of the responsive nerves *The disappearance of the colonic effects produced by central stimulation* after section of all the peripheral nerves strongly indicates a nervous and not a humoral transmission

Excitatory responses

(1) Parasympathetic pathways As judged from the transection studies the vagal nerves did not seem to mediate any detectable colonic influence either from the hypothalamus or from the mesencephalon Similar results were reported by Wang *et al (1940) in the intact animal whereas colonic responses produced by stimula*tion of the hypothalamus at the infundibular level were abolished by vagotomy in ? of 7 chronic spinal cats

With regard to the gastric motility the vagal nerves have been found to convey excitatory effects from the hypothalamus (Beattie and Sheehan 1934 Fennegan & Puiggari 1966) whereas Eliasson (1954) concluded that these effects as well the responses from the mesencephalon were mediated through cholinergic fibres in the splanchnic nerves *The present experiments fail to give evidence for a para*sympathetic pathway to the colon from the mesencephalon

The importance of the sacral parasympathetic outflow in mediation of impulses from forebrain structures to the lower colon following transection of the spinal cord at the 6th lumbar segments in cats has been demonstrated (Ingersoll and Jones 1946) The present experiments support these findings and clearly demonstrate that excitatory effects from the hypothalamus to the whole colon are *mediated through the pelvic nerves The blocking effect of atropine* and the failure to influence the response by guanethidine indicates that the fibres are cholinergic This is supported by the finding that atropine partly blocks the effects of direct pelvic nerve stimulation (Rostad 1973 b)

(2) Sympathetic pathways With respect to its influence on the colonic motility the sympatho inhibitory area differed from the other excitatory hypothalamic zones in three ways the response was associated with a fall in blood pressure it was *blocked by propranolol* and it was abolished by section of the lumbar colonic nerves

Concerning the gastric motility responses elicited from the sympatho-inhibitory area were seen only on a background of activity induced by vagal nerve stimulation (Jansson Lisander and Martinson 1969) In the present study however augmen a

tion of colonic motility could be induced from the sympatho inhibitory area without such simultaneous stimulation of the parasympathetic nerves Thus contrary to the stomach the colon seems to be under an inhibitory influence through the sympathetic nerves as evidenced by the conspicuous activity following section of these nerves (Rostad 1973 b) Therefore the increased motility and associated blood pressure falls obtained from this zone also after section of the parasympathetic nerves most likely are due to a sympatho inhibitory effect This is supported by the elimination of the response by propranolol and phenoxybenzamine which block beta and alpha inhibitory receptors respectively and which are known to be present in the intestinal wall (Lee 1970) The failure to elicit the response from the sympatho-inhibitory area when only the splanchnic nerves were intact presumably finds its explanation in the weak inhibitory influence exerted by these nerves (Rostad 1973 b) Finally the abolition of the colonic effects evoked from the sympatho-inhibitory area by selective section of the lumbar colonic nerves indicates that the latter constitutes the main pathway

The colonic excitatory effects associated with a rise in blood pressure induced from the other hypothalamic and mesencephalic areas were similarly partly conveyed through the lumbar colonic nerves These are known to contain motor fibres possibly cholinergic (see Rostad 1973 b) However the effects were not blocked by atropine but by guanethidine indicating an adrenergic transmission The concomitant blood pressure rise indicates an enhanced adrenergic discharge and not a sympatho-inhibition Furthermore the colonic effects produced from these areas were not blocked by a beta blocker but by an alpha blocker thus excluding the possibility of sympatho-inhibition *In vitro* studies of the rat colon have shown that stimulation of alpha receptors results in contraction of the smooth muscle (Gagnon and Belisle 1970) Furthermore alpha-excitatory receptors have been found in the longitudinal muscle of the guinea pig ileum (Lee 1970) The presence of such receptors in the colonic wall of the cat might explain the motility responses obtained from the excitatory hypothalamic and mesencephalic areas after section of the parasympathetic nerves

Inhibitory responses

The results of stimulation of the inhibitory hypothalamic and mesencephalic areas following peripheral nerve sections suggest that the effects are mediated through the lumbar colonic as well as through the splanchnic nerves However the responses conveyed by the latter appear to be weak and are mainly restricted to the proximal part of the colon This is in accordance with the fact that these nerves have their predominant inhibitory influence on the proximal colon (Rostad 1973 b)

The adrenergic nature of the inhibitory fibres as indicated by the blocking effect of guanethidine corresponds to the results of stimulating the lumbar colonic nerves after administration of autonomic blocking agents (see Rostad 1973 b)

The elimination of the inhibitory colonic responses after section of the splanchnic and lumbar colonic nerves is in agreement with the findings that neither the

nor the pelvic nerves have been found to contain inhibitory fibres (Hulten 1969 Rostad 1973 b)

In *conclusion* the excitatory colonic effects induced from all responsive hypothalamic and mesencephalic areas seem to be conveyed through adrenergic fibres within the sympathetic lumbar colonic nerves Cholinergic fibres in the parasympathetic pelvic nerves represent an additional pathway for effects from the excitatory hypothalamic areas with the exception of the sympatho inhibitory zone The inhibitory effects on the colonic motility produced by the inhibitory hypothalamic and mesencephalic areas are dependent on adrenergic fibres within the splanchnic and lumbar colonic nerves

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References

BEATTIE J and D SHEEHAN The effects of hypothalamic stimulation on gastric motility *J Physiol* (Lond) 1934 *81* 218—227

ELIASSON S Activation of gastric motility from the brain stem of the cat *Acta physiol scand* 1954 *30* 199—214

FENNEGAN F M and M J PUIGGARI Hypothalamic and amygdaloid influence on gastric motility in dogs *J Neurosurg* 1966 *24* 497—504

FOLKOW B B JOHANSSON and B ÖBERG A hypothalamic structure with a marked inhibitory effect on tonic sympathetic activity *Acta physiol scand* 1959 *47* 262—270

GAGNON D J and S BELISLE Stimulatory effects of catecholamines on the isolated rat colon after beta adrenergic blockade with oxprenolol and propranolol *Europ J Pharmacol* 1970 *2* 303—309

HULTEN L Extrinsic nervous control of colonic motility and blood flow *Acta physiol scand* 1969 Suppl 335

INGERSOLL E H and L JONES The effect upon the colon of electrical stimulation of forebrain areas in the cat *Amer J Physiol* 1946 *146* 187—191

JANSSON G B LISANDER and J MARTINSON Hypothalamic control of adrenergic outflow to the stomach in the cat *Acta physiol scand* 1969 *75* 176—186

LEE C Y Adrenergic receptors in the intestine In p 549—557 in Bulbring E A Brading A Jones and T Tomita (eds) *Smooth muscle* London E Arnold Ltd 1970

ROSTAD H Colonic motility in the cat I Extraluminal strain gage technique Influence of anesthesia and temperature *Acta physiol scand* 1973 a *89* 79—90

ROSTAD H Colonic motility in the cat II Extrinsic nervous control *Acta physiol scand* 1973 b *89* 91—103

ROSTAD H Colonic motility in the cat III Influence of hypothalamic and mesencephalic stimulation *Acta physiol scand* 1973 c *89* 104—115

ROSTAD H Colonic motility in the cat V Influence of telencephalic stimulation and peripheral pathways mediating the effects *Acta physiol scand* 1973 d *89* 169—181

WANG S C G CLARK F L DEY and S W RANSON Further study on the gastro-intestinal motility following stimulation of the hypothalamus *Amer J Physiol* 1940 *130* 81—88

Acta physiol scand 1973 89 169—181
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Colonic Motility in the Cat

V Influence of Telencephalic Stimulation and the Peripheral Pathways Mediating the Effects

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Abstract

Rostad H *Colonic motility in the cat V Influence of telencephalic stimulation and the peripheral pathways mediating the effects* Acta physiol scand 1973 *89* 169—181

The cerebral cortex and intracerebral structures were stimulated in lightly anesthetized cats while colonic motility was recorded using extraluminal strain gage transducers Excitatory colonic responses were produced from the anterior ectosylvian gyrus (the second somatic sensory motor area) and from a small zone around the anterior end of the lateral sulcus These effects were shown to be mediated through the vagal nerves Colonic inhibition resulted from stimulation of three separate cortical zones the anterior sigmoid the orbital and the anterior cingulate gyri These effects were mediated mainly through the lumbar colonic nerves Stimulation of the amygdaloid nuclei produced excitatory colonic effects mediated through the lumbar colonic nerves Inhibitory effects were not obtained from the amygdala Finally weak colonic contractions resulted from stimulation of the olfactory bulb and tract and were found to be conveyed through the parasympathetic vagal and pelvic nerves

In preceding reports the effects of hypothalamic and mesencephalic stimulation on the colonic motility of the cat (Rostad 1973 c) as well as the peripheral pathways mediating these effects (Rostad 1973 d) have been studied Such information on the influence of telencephalic structures on colonic motility is sparce

Concerning the *cerebral cortex* Watts and Fulton (1934) observed a marked increase in peristaltic activity of the coecum and lower part of the small intestine following stimulation of the premotor frontal area in monkeys Both excitatory and inhibitory effects on colonic motility were obtained from the premotor cortex of dogs (Spiegel Weston and Oppenheimer 1943)

In cats anesthetized with chloralose Strom and Uvnas (1950) observed inhibition of small intestinal peristalsis on stimulating a point close to the bottom of the cruciate sulcus and colonic contractions were produced from points in the genual portion of the anterior cingulate gyrus

In chronic experiments on dogs, stimulation of the anterior cingulate gyrus caused inhibition of peristalsis of the stomach and small intestine followed by contractions (Bogach and Koval 1968) Stimulation of the same area while the stomach and small intestine were in a state of rest often stimulated their motor activity

The augmentatory as well as inhibitory influence of the amygdaloid nuclei on gastric motility is well established (for references see Kaada 1972) The activity of the small intestine is also affected stimulation of the parvocellular basal nuclei in anesthetized cats produced inhibition of its peristaltic contractions (Koikegami 1964) In chronic experiments on dogs amygdaloid stimulation produced either inhibition *or facilitation* depending on the state of motor activity of the gut (Bogach and Koval 1968) The influence of the amygdala on the colonic motility has not been previously studied

Electrical stimulation *of the olfactory bulb and tract has produced inhibitory* effects on gastric motility in cats while the colon remained uninfluenced (Strom and Uvnas 1950)

The septal area is also known to influence various autonomic functions including induction of defecation (Hess 1954 pp 12—24)

The *purpose* of the present work which represents a continuation of preceding investigations (Rostad 1973 c d) was to study the influence of telencephalic structures on colonic motility particularly those known to influence other types of autonomic activities Various cortical areas on the lateral medial and ventral aspects of the hemispheres the amygdala the olfactory bulb and tract and the septal nuclei have been electrically stimulated and colonic motility recorded by sensitive extraluminal strain gage transducers described previously (Rostad 1973 a)

The peripheral pathways mediating the effects have been traced by section of various colonic nerves and by combined central and extrinsic nerve *stimulation* as described in a preceding report (Rostad 1973 d)

Material and methods

The results are based on data gained from [illegible]0 cats mostly male weighing between [illegible] and 5 6 kg The preoperative regime was as described previously (Rostad 1973 a) Most animals were anesthetized with pentobarbital sodium (Nembutal® Abbott) 30 mg/kg b w i p and some with chloralose 30—40 mg/kg b w i v after induction with ether In addition to chloralose one animal was given urethane 400 mg/kg b w and in a few experiments a combination of barbiturate and chloralose anesthesia was used The neuromuscular blocking agent pancuronium bromide (Pavulon® Organon) was administered in amounts of 0 1—0 3 mg/kg b w i v in order to prevent striated muscle artifacts thus permitting the use of lightly anesthetized animals

The operative procedures and *recording techniques* were as described previously (Rostad 1973 a) In brief the colonic motility of the proximal the middle and *the lower part* of the colon was *recorded* as well as arterial blood pressure and respiratory movements The catecholamines from the adrenal glands were excluded from the circulation by careful ligation of the vessels of both glands Adrenocortical substitution was secured by i v injection of hydrocortisone (Solu-Cortef® Upjohn) 5—10 mg/kg b w

Electrical stimulation of the brain The head of the animal was immobilized in a Horsley-Clarke frame. *Craniotomy was performed* and the dura removed the cerebral cortex being exposed to the extent required To reach the orbital surface of the hemisphere one eye was enucleated Exposure *of the* olfactory bulb required removal of the frontal sinus and part of

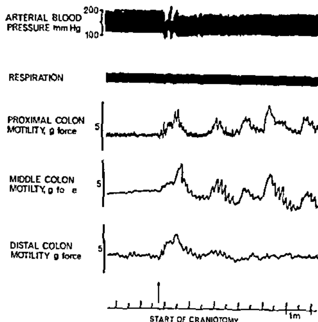


Fig 1 Cat 4 2 kg Effects on the colonic motility of craniotomy Start of craniotomy at arrow

the posterior ethmoidal cells The medial cortical surface was uncovered by removal of part of the opposite hemisphere by gentle suction In some experiments it was reached stereotaxicall The cortex was usually *repeatedly covered with mineral oil at a temperature of 38 C to* minimize cooling and drying and spread of the stimulating current

For intracerebral stimulation a monopolar or occasionally a concentric bipolar stainless steel electrode (previously described Rostad 1973 c) was stereotaxically inserted using the coordinates given by Jasper and Ajmone Marsan (1954)

The cerebral cortex was stimulated through small bipolar ball tipped electrodes with an interelectrode distance of about 1—2 mm The surface of the olfactory bulb and tract was stimulated in the same way while the interior of these structures was reached by a monopolar or bipolar concentric electrode

Electrical square wave pulses at frequencies from 5—200 Hz and of 1—10 ms duration were employed The intensity varied from 1—5 V

The *histological* identification of the electrode placements *extr c erie sect on nd timulation* were performed as described in previous reports (Rostad 1973 b c)

Results

The colonic motility was usually not influenced by craniotomy performed over the posterior half of the hemispheres However on removal of the frontal sinus and the ethmoidal cells a considerable increase in motility lasting for 10—12 min was observed in 12 of 18 expts (Fig 1) This temporary effect is presumably due to mechanical stimulation of the cerebral cortex and olfactory bulbs

Stimulation of cerebral structures resulted in both excitatory and inhibitory colonic responses An excitatory effect could never be reversed into an inhibitory one or *vice versa* by varying the stimulus parameters Similarly the effects of brain sti

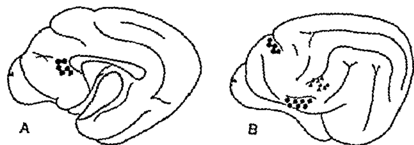


Fig 2 Colonic responses on stimulation of the cerebral cortex in cats *A* Medial aspect of the cerebral hemisphere *B* Lateral aspect Triangles Excitatory colonic responses Large filled circles Inhibitory effects Small dots No response

lation did not appear to depend on the tonal condition of the colon The optimal parameters for cortical stimulation were 10—50 Hz at 3—5 ms and for intracerebral stimulation 80—100 Hz at 1 ms

A The cerebral cortex

The cerebral cortex including most of its lateral medial and ventral anterior aspects was stimulated in 20 animals The responsive areas are indicated in Fig 2 Weak excitatory colonic responses on the proximal half of the colon were obtained from two distinct areas in the parietal region i e in the anterior end of the anterior ectosylvian gyrus apparently corresponding to the second somatic sensory motor area (SII) of Woolsey (1958) and in a small zone around the anterior end of the lateral sulcus The responses were characterized by slow sustained contractions with rhythmic waves superimposed (Fig 3 A)

Inhibition of all colonic areas was elicited by stimulation of three separate zones i e in the anterior sigmoid the orbital and the anterior cingulate gyri (Fig 4 A B) No significant differences between these three areas were observed with respect to stimulus thresholds or in the character of the responses

The cortical effects on the blood pressure were small under the present conditions and with the stimulus intensity used On the whole the cortical responses were usually weaker and more inconsistent and the latent period was usually a few seconds longer than that seen with stimulation of diencephalic and mesencephalic structures (Rostad 1973 c) The effects were best seen with penthiobarbital anesthesia Under chloralose the effects were weaker and under urethane no significant colonic responses were obtained

The validity of the cortical responses was demonstrated by their abolition following local cortical application of lidocaine (Xylocain® 1 % Astra) The effects are therefore not due to physical spread of current to subcortical structures or to the meninges

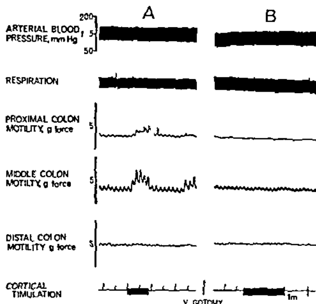


Fig 3 Cat 2 6 kg Weak excitatory responses in the proximal part of the colon on stimulation of the anterior ectosylvian gyrus (A) The effects are abolished by bilateral cervical vagotomy (B) Stimulus parameters 15 Hz 3 ms 6 V

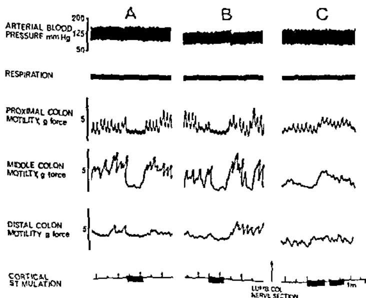


Fig 4 Cat 2 9 kg Inhibitory colonic responses on stimulation of the anterior sigmoid gyrus (A) and anterior cingulate gyrus (B) The effects from both ones are abolished by section of the lumbar colonic nerves (C) Stimulus parameters 65 Hz 1 ms 6 V

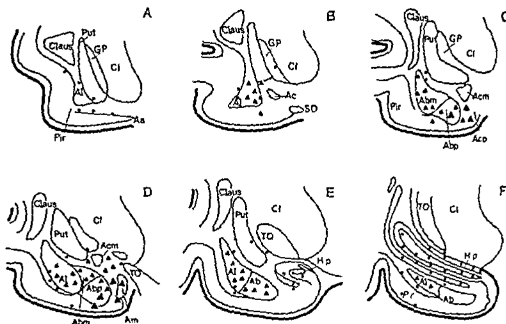


Fig 5 Diagram of frontal sections in rostro-caudal direction (A—F) through the amygdaloid nuclear complex of the cat indicating *representative points which on electrical stimulation influenced the colonic motility* Triangles Excitatory *colonic responses* Large symbols indicate the most sensitive points Small dots No response Outline of drawings from Ursin and Kaada (1960)

Abbreviations (Nomenclature according to Jasper and Ajmone Marsan 1954)

a area amygdaloidea anterior
n amygdaloideus basalis
n amygdaloideus basalis
(pars magnocellularis)
bp n amygdaloideus basalis
(pars parvocellularis)
Ac n amygdaloideus centralis
Acm n amygdaloideus centralis
(pars medialis)
Aco n amygdaloideus corticalis
Al n amygdaloideus lateralis
Am n amygdaloideus medialis
CI capsula interna
Claus claustrum
GP globus pallidus
Hip hippocampus
Pir lobus piriformis
Put putamen

B The amygdala and olfactory bulb and tract

The amygdaloid nuclear complex was stimulated in 23 animals with about 700 stimulations Representative responsive and non responsive points are illustrated in Fig 5 In general the amygdaloid effects were less marked and less consistent than those obtained from the hypothalamus (Rostad 1973 c) The best effects were produced from the medial and cortical nuclei and the parvocellular part of the basal nuclei (Fig 6 A) From these regions a significant increase of motility in all parts of the colon was regularly obtained usually associated with a slight decrease or rise in blood pressure Weak colonic motor responses also resulted from stimulation of the lateral the central and the magnocellular part of the basal nuclei

Clearcut inhibitory effects on the colonic motility were not induced from the amygdala although extensive areas of the amygdaloid region were thoroughly searched with various stimulation parameters in a number of animals

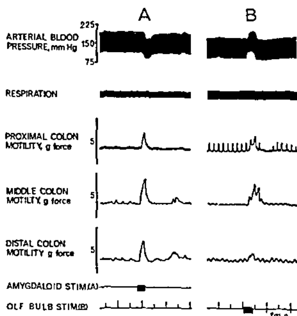


Fig 6 *A* Cat 3 5 kg Excitatory colonic responses on stimulation of the amygdala (80 cps 1 ms 3 V) *B* Cat 3 kg Excitatory colonic responses most conspicuous in the proximal part on stimulation of the olfactory bulb (50 Hz 1 ms 4 V)

Stimulation of *the olfactory bulb and tract* (14 animals) produced weak contractions of the colon most conspicuous in its proximal half (Fig 2 and Fig 6 B) Inhibitory responses were never observed The associated effects on the blood pressure varied from animal to animal but was usually augmentatory

TABLE I Effects on colonic motility of telencephalic stimulation after bilateral section of various extrinsic nerves as indicated in the left column Figures within parenthesis The number of animals stimulated Figures outside parenthesis The number of these animals in which the excitatory or inhibitory responses from the area indicated on the top were obtained Framed figures No response

Nerves sectioned		Area stimulated			
		Excitatory responses			Inhibitory responses
		Anterior ectosylvian gyrus	Amygdala	Olfactory bulb/tract	Anterior sigmoid orbital and anterior cingulate gyri
Vagal	(7)	0 (3)	6 (6)	3 (3)	2 (2)
Lumbar colonic	(6)	2 (2)	0 (5)	1 (1)	2 (4)
Pelvic	(6)	2 (2)	3 (3)	3 (3)	2 (2)
Vagal and pelvic	(16)	0 (3)	10 (11)	0 (4)	5 (5)
Lumbar colonic and splanchnic	(6)	1 (1)	0 (4)	1 (1)	0 (2)
Vagal pelvic and lumbar colonic	(5)	0 (1)	0 (2)	0 (1)	? (4)

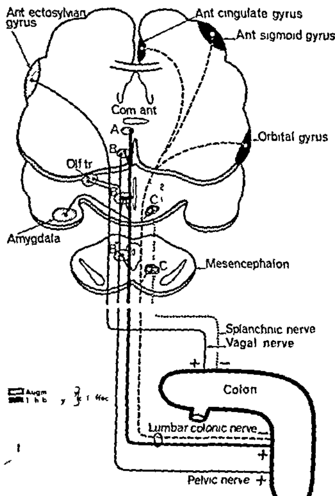


Fig 7 Schematic drawing indicating the peripheral pathways mediating the effects on colonic motility induced by stimulation of various cerebral areas in the cat Left augmentatory and right inhibitory responses *A* Hypothalamic sympatho-inhibitory area Augmentatory colonic effects with a concomitant fall in blood pressure *B* and *B'* Hypothalamic and *B'* mesencephalic areas Increase of colonic motility associated with a rise in blood pressure *C* Hypothalamic and *C'* mesencephalic areas from which colonic inhibition was obtained

C The septal nuclei

The septal nuclei were stimulated in 8 cats The effects on the colonic motility and blood pressure were small and inconsistent In 2 animals a weak contraction in the lower part of the bowel was observed

Peripheral pathways

The results of telencephalic stimulation following bilateral nerve sectioning are summarized in Table I The schematic drawing in Fig 7 indicates the principal peripheral pathways found to mediate the colonic influence following cerebral stimulation The diagram also includes the peripheral pathways responsible for the effects produced on stimulating hypothalamic and mesencephalic areas (Rostad 1973 c d)

Because of the weak and sometimes inconsistent colonic effects from *cerebral cortical* stimulation the peripheral pathways conveying the effects from these areas were difficult to determine However the excitatory colonic responses from the

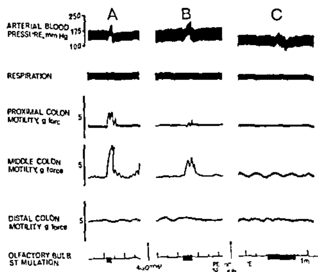


Fig 8 Cat 3 7 kg *A* Excitatory responses in the proximal part of the colon on stimulation of the olfactory bulb (10 Hz 3 ms 3 V) *B* The effects are weaker after bilateral cervical vagotomy in spite of higher stimulus intensity (10 Hz 3 ms 5 V) *C* Abolition of the responses by bilateral section of the pelvic nerves

anterior ectosylvian gyrus were abolished by bilateral vagotomy (Fig 3 B) while section of the other extrinsic nerves selectively or in combination left the effects unchanged (Table I) *Thus the effects from this cortical areas seem to be mediated* through the vagal nerves

The inhibitory colonic effects from the anterior sigmoid the orbital and the anterior cingulate gyri persisted after section of the vagal and pelvic nerves as well as after section of both these nerves together Selective section of the lumbar colonic nerves abolished *or greatly reduced the effects from the inhibitory cortical zones* (Fig 4 C) Stimulation of the orbital and anterior cingulate gyri produced weak inhibitory colonic effects in 2 animals Inhibitory responses were not obtained when the lumbar colonic as well as the splanchnic nerves had been divided whereas the inhibition persisted following section of both the vagal and pelvic nerves

Thus *the inhibitory colonic effects induced by cortical stimulation seem to be* mediated mainly through the lumbar colonic and partly through the splanchnic nerves

Stimulation of *the amygdaloid nuclear complex* after section of various extrinsic nerves produced colonic effects only when the lumbar colonic nerves were left intact indicating that these nerves are mediating the effects (Table I) Inhibitory effects could not be obtained although abundant background colonic motility was induced by just suprathreshold stimulation of the peripheral ends of the cut vagal or pelvic nerves

The excitatory responses from *the olfactory bulb and tract* still persisted after selective section of the vagal or pelvic nerves respectively (Table I) However the effects were weaker and required higher stimulus intensity (Fig 8 A B) However when both the vagal and pelvic nerves had been cut the colonic responses

abolished (Fig 8 C) indicating that each of these nerves conveys excitatory effects from the olfactory bulb and tract and that it is necessary to remove the summated effects of these two sets of nerves to prevent the influence from these structures

Discussion

The present experiments demonstrate that colonic motility is influenced from the same telencephalic regions that are known to control other autonomic activities The responses to stimulation were usually weak and the peripheral pathways mediating the effects were in part different from those of the hypothalamus and mesencephalon (Rostad 1973 d)

Cortex cerebri A group of cortical points yielding *excitatory* colonic responses are located in the region corresponding to the second somatic sensory motor area (SII) of Woolsey (1958) The same area has been found to yield augmentatory and inhibitory effects on the bladder motility in cats (Gjone and Setekleiv 1963) In man electrical stimulation of the second somatic sensory motor area gives rise to abdominal and epigastric sensations and a concomitant change in gastric motility (Penfield and Faulk 1955) Attention should be paid to the recent observation that this cortical area projects to the lateral amygdaloid nucleus (Lescault 1971) an area which in the present study similarly was found to produce increased colonic contractions

The vagal nerves were found to be the principal mediator of the augmentatory colonic effects produced by stimulation of the second somatic sensory motor area Evoked potentials have been recorded from the same cortical area following afferent vagal stimulation (Aubert and Legros 1963)

The small zone in the parietal area which produced excitatory colonic effects seems to correspond to the area from which Eliasson (1952) produced increase in gastric motility in cats Because of the relatively weak and inconsistent colonic responses the peripheral pathways mediating the effects from this zone could not be determined The zone appears to correspond to that part of the first sensory motor area from which evoked potentials have been recorded following afferent splanchnic and mesenteric nerve stimulation (Amassian 1951 Downman 1951 Gardner Thomas and Morin 1955)

Inhibitory colonic responses were regularly elicited from the anterior sigmoid gyrus Similar effects on the gastric motility in the cat were described by Eliasson (1952) and Hesser and Perret (1960) while contraction of the small intestine was obtained following stimulation of apparently the corresponding cortical zone in dogs and monkeys (Bochefontaine 1876 Watts and Fulton 1934 Davey Kaada and Fulton 1950)

The anterior cingulate and orbital gyri both causing inhibition of colonic motility when stimulated have been included in the limbic structures (Kaada 1951 1960) The colonic inhibition evoked from the pregenual part of the anterior cingulate region is in accordance with the widespread inhibition of somatomotor and autonomic

activities exerted by this zone including depression of the motility of the stomach blood pressure (see Kaada 1960) the urinary bladder (Gjone and Setekleiv 1963 and the uterus (Setekleiv 1964) In the present experiments the effects on the blood pressure and pulse rate were less marked than in several previous studies presumably because of the stabilizing effect of pancuronium bromide (Rostad 1973 b)

The colonic inhibition induced from the three cortical areas appears to be mediated through the lumbar colonic and splanchnic nerves The intracerebral path ways are not known However attention should be paid to the projection of fibres from the posterior orbital and premotor cortex (area 6) to the ventromedial hypo thalamic nucleus This connection is bilateral and passes through the preoptic region to reach the ventromedial nucleus by way of the medial forebrain bundle (Le Gros Clark and Meyer 1950) The latter authors considered this nucleus as an important focal area for a number of fibre systems concerned with the control of autonomic functions The cingulate gyrus indirectly influences the hypothalamus via the hippo campus and the so-called limbic system (Raisman 1966) Since stimulation of points within the medial forebrain bundle as well as in the ventral part of the ventromedial hypothalamic nucleus is known to produce colonic inhibition which similarly appears to be mediated through the lumbar colonic and splanchnic nerves the presence of an inhibitory cortico hypothalamic peripheral sympathetic system is indicated

The inhibitory effects on *gastric* motility induced by stimulation of the same three cortical areas appears to be mediated through the same intracerebral route (Elias on 1952) However this correspondance apparently does not apply to the pe ripheral pathways as the inhibition of gastric motility seems to be dependent on the vagal nerves This has been demonstrated for the anterior cingulate (Babkin and Kite 1950 Kaada 1951 Hesser and Perret 1960) the orbital (Kaada 1951 Eliasson 1952 Hesser and Perret 1960) as well as for the anterior sigmoid gyri (Eliasson 1952 Hesser and Perret 1960) Thus the inhibition of motility of the proximal and distal part of the gastrointestinal tract induced from apparently the same cortical zones seems to follow different peripheral pathways

Amygdaloid nuclear complex Stimulation of this region results in complex somatic visceral endocrine and behavioural effects (reviewed by Kaada *1972*) However augmentation of colonic motility has to the author s knowledge not been described previously With regard to alimentary behaviour stimulation of the same areas which in the present experiments produced optimal effects viz the cortical and medial nuclei and the adjoining part of the parvocellular part of the basal nucleus has been found to result in increased food intake (Lewinska 1968) whereas stimulation of the basolateral part of the amygdala inhibits food intake Removal of the corresponding areas produces the opposite effects a decrease and an increase in food intake respectively (Lewinska 1967) In the present study a corresponding inhibition of colonic motility following stimulation of the basolateral part of the nuclear complex could not be found The results regarding the localization of amygdaloid areas exciting and inhibiting gastric motility are not unequivocal (*cf* Kaada 1972)

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ELIASSON S Cerebral influence on gastric motility in the cat *Acta physiol scand* 1952 Suppl 95

GARDNER E D L M THOMAS and F MORIN Cortical projections of fast visceral afferents in the cat and monkey *Amer J Physiol* 1955 *183* 438—444

GJONE R. and J SETEKLEIV Excitatory and inhibitory bladder responses to timulation of the cerebral cortex in the cat *Acta physiol scand* 1963 *59* 337—348

HESS W R *Diencephalon Autonomic and extrapyramida functions* New York Grune & Stratton 1954

HESSER F H and G E PERRET Studies on gastric motility in the cat II Cerebral and infra cerebral influence in control vagectomy and cervical cord preparations *Gastroenterology* 1960 *38* 231—246

JASPER H H and C AJMONE MARSAN *A stereota ic atlas of the diencephalon of the cat* Ottawa, National Research Council of Canada 1954

KAADA B R Somato-motor autonomic and electrocorticographic responses to electrical stimu lation of "rhinencephalic and other structures in primates cat and dog *Acta physiol scand* 1951 Suppl 83

KAADA B R Cingulate posterior orbital anterior insular and temporal pole cortex Pp 1345 —1372 in *Handbook of Physiology* Sect. 1 Vol 2 Baltimore Williams and Wilkins Co 1960

KAADA B R *Stimulation of the amygdaloid complex with reference to functional representa tions* Pp 205—281 in Eleftheriou B E (ed) *The neurobiology of the amygdala* New York London Plenum Press 1972

KOIKEGAMI, H Amygdala and other related limbic structures experimental studies on the anatomy and function *Acta med biol* (Niigata) 1964 *12* 3—266

LESCAULT H Some neocortico-amygdaloid connections in the cat Thesis University of Ottawa, 1971

LEWINSKA M K. Changes in eating and drinking produced b partial amygdalar lesions in cat *Bulletin of the Polish Academy of Science* (Biology) 1967 *15* 301—305

LEWINSKA M K. *Inhibition and facilitation of alimentary behavior elicited by stimulation of* amygdala in the cat *Acta Biol exp* (Warszawa) 1968 *28* 23—34

PENFIELD W and M E FAULK JR. The insula *Brain* 1955 *78* 445—470

RAISMAN G Neural connexions of the hypothalamus *Brit med Bull* 1966 *22* 197—201

ROSTAD H Colonic motility in the cat I Extraluminal strain gage technique Influence of anesthesia and temperature *Acta physiol scand* 1973 a *89* 79—90

ROSTAD H Colonic motility in the cat. II Extrinsic nervous control *Acta physiol scand* 1973 b *89* 91—103

ROSTAD H Colonic motility in the cat III Influence of hypothalamic and mesencephalic stimulation *Acta physiol scand* 1973 c *89* 104—115

ROSTAD H Colonic motility in the cat IV Peripheral pathways mediating the effects in duced by hypothalamic and mesencephalic stimulation *Acta physiol scand* 1973 d *89* 154 —168

SETEKLEIV J Uterine motility of the estrogenized rabbit V Response to brain stimulation *Acta physiol scand* 1964 *62* 313—322

SPIEGEL E A K WESTON and M J OPPENHEIMER Postmotor foci influencing the gastro-intestinal tract and their descending pathways *J Neuropath exp Neurol* 1943 *2* 45—57

STRÖM G and B UVNÄS Motor responses of gastro-intestinal tract and bladder to topical stimulation of the frontal lobe basal ganglia and hypothalamus in the cat *Acta physiol scand* 1950 *21* 90—104

URSIN H and B R KAADA Functional localization within the amygdaloid complex in the cat *Electroenceph clin Neurophysiol* 1960 *12* 1—20

WATTS J W and J F FULTON Intussusception — the relation of the cerebral cortex to in testinal motility in the monkey *New Engl J Med* 1934 *210* 883—896

WOOLSEY C N Organization of somatic sensory and motor areas of the cerebral cortex Pp 63—81 in Harlow H F and C N Woolsey eds *Biological and biochemical basis of behavior* Madison The University of Wisconsin Press 1958

Acta physiol scand 1973 89 182—186

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The Count of Mast Cells, Tissue Eosinophils, and Epithelial Mitoses of Rat Gastrointestinal Mucosa after Aspirin Treatment

By

Toivo Räsänen and Eero Taskinen

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Abstract

Räsänen T and E Taskinen *The count of mast cells tissue eosinophils and epithelial mitoses of rat gastrointestinal mucosa after aspirin treatment* Acta physiol scand 1973 *89* 182—186

Mucosal mast cells tissue eosinophils and epithelial mitoses were counted in the gastrointestinal tract of rats given 1 3 and 5 times 10 mg of aspirin in 5 ml of water at 12 h intervals by cannulation into the stomach The mast cell count of the gastric and duodenal mucosa of rats receiving aspirin fell sharply No significant change was observed in tissue eosinophils The mitotic count in the epithelium of stomach declined clearly but no decrease occurred in mitoses of the epithelial cells in the mucosa of the duodenum jejunum and colon The results seem to indicate that the injurious action of aspirin is transmitted so that the gastric mucosal mast cells possibly participate in it directly the heparin contained in the mast cell granules inhibits in the phase of liberation the mitotic activity especially in the gastric epithelium and the blood coagulation mechanism locally The histamine and 5-hydroxytryptamine liberation associated with the release of heparin results in the stimulation of secretion and in vascular reactions of the gastric mucosa

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When gastric mucosal bleeding is caused by aspirin or by salicylic acid the agent functions as a trigger for bleeding regardless of the mode of administration (Grossman Matsumoto and Lichter 1961) Shapiro Redish and *Campbell* (1915) observed that the bleeding mechanism caused by salicylic acid *resembles* a dicumarin like effect in the gastric mucosa

The structure of the cell membranes in the gastric epithelium changes under the influence of salicylates so that the reflux of potassium and hydrogen ions *through* the mucosa happens uncontrolled (Davenport 1965) *The acidity of the gastric* juice increases and its neutralisation decreases the hemorrhagic effect of *aspirin in* gastric mucosa (Davenport 1964 Overholt Brodie and Chase 1969 and *the non* selective mobility of ions through the epithelium (Davenport *et al* 1965 Overholt and Pollard 1968)

TABLE I The count ±S.E. of mucosal mast cells and epithelial mitoses in rat gastrointestinal tract after aspirin treatment

Mast Cells		Number of counts	Stomach	Duodenum	Jejunum	Colon
Aspirin	1×10 mg	8	71±11	155±45		
	3×10 mg	8	87±19	224±28		
	5×10 mg	7	20±11	59±11		
Controls	3× 5 ml of saline	8	249±30	311±51		
Epithelial mitoses						
Aspirin	1×10 mg	8	43± 4	277±18	263±15	65±6
	3×10 mg	8	21± 5	253±14	219±25	50±7
	5×10 mg	7	41± 6	315±45	229±24	51±7
Controls	3× 5 ml of saline	8	91± 4	310±19	274±27	70±8

In addition to epithelial and vascular damage the blood coagulation mechanism decided therefore to study the aspirin induced changes in the mucosal mast cells that is heparinocytes tissue eosinophilia and epithelial mitoses of the rat gastro intestinal canal

Method

Male rats of Sprague Dawley strain aged about 5 months were used in the study They were given 2 weeks to adopt to laboratory conditions before the experiment During this time and throughout the experiment the rats were given a normal standardised rat laboratory diet (Hankkija) and water *ad lib* Aspirin dissolved in water was introduced into the stomach with a cannula dose 10 mg/5 ml

The procedure was as follows

Group I 1×10 mg of aspirin
Group II 3×10 mg of aspirin at intervals of 12 h
Group III 5×10 mg of aspirin at intervals of 12 h
Group IV 3×5 ml of saline at intervals of 12 h

5 h after the last cannulation the rats were exsanguinated under light ether narcosis and samples were taken immediately from the wall of the stomach duodenum ileum and colon Pieces of the tissues were fixed in Bouin's solution and in fresh alkaline lead acetate Section 4 μm in thickness were cut from the blocks made by Bouin's fixation and stained with hemalaun eosin for the count of mitoses and eosinophilic leucocyte 10 μm thick sections from fixation in the 4 % lead acetate were stained with toluidin blue for the counting of the mucosal mast cells

Using Leitz's plane wide angle ocular objective system and magnification ×500 gastric mucosal mast cells and mitoses were counted in the superficial corpus mucosa of the glandular stomach from 20 successive visual fields in the basal part of the intestinal mucosa from 10 visual fields Eosinophilic leucocytes were counted separately in the superficial and basal gastric mucosa from 10 visual fields and in the basal duodenal mucosa The significance of the differences was analysed by means of Fisher Student's t test

Results

Mucosal mast cells were degranulated by aspirin to such an extent that the number of granulated cells after a single dose of aspirin was significantly lower than in the gastric mucosa of the control rats (Table I) The number of the mucosal mast

TABLE II The count ±S E of eosinophilic leucocytes in rat gastric and duodenal *lamina propria* after aspirin treatment

Treatment		Gastric mucosa		Duodenal mucosa
		superficial	basal	
Aspirin	1 × 10 mg	19 ± 4	457 ± 41	451 ± 32
Aspirin	3 × 10 mg	63 ± 35	476 ± 74	471 ± 36
Aspirin	5 × 10 mg	27 ± 10	319 ± 74	295 ± 41
Controls		34 ± 7	480 ± 89	409 ± 31

on the third day declined further in both the gastric and the intestinal mucosa of the animals given aspirin continuously

The count of epithelial mitoses in the gastric mucosa showed a sharp and highly significant drop during aspirin treatment On the other hand changes in the epithelial mitoses were slight in the intestinal epithelium and not statistically significant

In tissue eosinophilia no clearly demonstrable changes were seen in the superficial or basal part of the gastric mucosa and in the intestinal mucosa (Table II)

Discussion

The number of the gastric mucosal mast cells was lower than the number of the duodenal mucosal mast cells in the control animals of this study This is possibly due to the obvious local irritation of the stomach and due to the systemic stress caused by the cannulation This probably explains the higher number of the mast cells in the intestinal than in the gastric mucosa contrary to the earlier observations (Räsänen 1963)

Gastric mucosal mast cells undergo degranulation faster than mast cells in the intestinal mucosa under the influence of aspirin a phenomenon that also has been observed in connection with glucocorticoid action Complete degranulation of mucosal mast cells results in the inhibition of gastric secretion and in the disappearance of the lesion production during reserpine administration (Räsänen 1971) According to Gottschalk and Menguy (1970) the lesion inducing effect of aspirin disappears in the gastric mucosa of rat during post radiation achlorhydria In an unpublished study whole body irradiation with 700 r resulted in complete loss of metachromasia from mast cell granules in the gastric and intestinal mucosa of rats (Holsti Räsänen and Cederberg 1966) The protective effect of radiation against aspirin induced mucosal bleeding may be based on the advanced degranulation of mucosal mast cells

Aspirin causes bleeding even without the co-operation of hydrochloric acid in human beings with atrophic gastric epithelium (John and Mc Dermott 19[illegible]) It contains however fairly numerous mucosal mast cells (Räsänen 1958) Cerand

(1969) reported a decrease in the number of gastric epithelial mitoses in connection with ulcerogenesis provoked by various agents This may be due to the specific action of both endogenous and exogenous heparin on the epithelium of gastric mucosa (Rasanen Cederberg and Taskinen 1966) The chain of events in the gastric epithelium during the influence of aspirin is probably the same—antimitotic action of endogenous heparin The observation that the healing of gastric lesions in dogs is retarded by aspirin (Sugawa Lucas and Walt 1970) probably rests on the same phenomenon

No manifest changes occurred in the mitotic count in the intestinal epithelium during aspirin treatment A similar observation has been made regarding the intestinal epithelium under the influence of exogenous heparin (Rasanen *et al* 1966) The polysaccharide isolated from rat intestinal mucosa differs in molecular structure from that of the gastric mucosa (Bianchini 1958)

No distinct changes of the kind seen under the influence of ACTH or glucocorticoids (Rasanen 1962) were observed in tissue eosinophilia of the gastric mucosa during aspirin effect It is perhaps evident that the changes in the mucosal mast cells and epithelial mitoses of the gastric wall are provoked directly by aspirin In the cell membrane salicylate immediately increases the cation permeability especially in regard to K ion (Levitan and Barker 1972)

The mechanism of the action of aspirin on the gastric mucosa is probably as follows Due to the nature of the gastric epithelium its ability to select the ion flows is disturbed by aspirin The gastric secretion (Clark and Adams 1947 Winkelman and Summerskill 1961) the histamine content in the venous blood of the stomach (John on and Overholt 1967) as well as the histamine forming capacity in the gastric mucosa (Johnson 1968) increases during this pha e The histamine probably derives from the degranulating mucosal mast cells a phenomenon associated with gastric secretory stimulation (Rasanen 1967)

Except the specific antimitotic effect on the gastric epithelium heparin has a local anticoagulating effect in the gastric mucosa An additional factor in the bleeding probably is the hyperemia during aspirin effect (Faggioli *et al* 1970) which may be due to the liberation of 5 hydroxytryptamine from mast cell granules Further in rat the lesion forming influence of aspirin on the gastric mucosa weakens after feeding (Brodie Tate and Hooke 1970) which primarily degranulates the mucosal mast cell (Rasanen 1964) and leads to a temporary loss of heparin before the aspirin protecting the mucosa for hemorrhagic lesions

References

BIANCHINI P Su di eparinoide della mucosa duodenale *Boll Soc ital Biol sp* 1958 *34* 685—688

BRODIE D A C L TATE and K F HOOKE Aspirin Intestinal damage in rats *Science* 1970 *170* 183—185

CLARK B B and W L ADAMS The effect of acetylsalicylic acid on gastric secretion Gastroenterology 1947 *9* 461—465

DAVENPORT H W Gastric mucosal injury by fatty and acetylsalicylic acids *Gastroente* 1964 *46* 245—253

DAVENPORT H W Potassium fluxes across the resting and stimulated gastric mucosa Injury by salicylic and acetic acids *Gastroenterology* 1965 *40* 238—245
DAVENPORT H W B J COHEN M BREE and V D DAVENPORT Damage to the gastric mucosa Effects of salicylates and stimulation. *Gastroenterology* 1965 *40* 189—196
FAGGIOLI, F G GASBARRINI G SCONDOTTO M MATTEI and A ZANETTI *Effetti dell a pirina* a del salicilato sulla mucosa gastrica. *G Clin med* 1970 *51* 298—319
GERARD A. Regeneration cellulaire dans la muqueuse gastrique du chien sous l effet de divers stimulants et de diverses drogues ulcerigenes *Biol Gastro Enterol* 1969 *58* 5—14
COTTSCHALK A and R. MENGUY Role of gastric acid in aspirin induced erosive gastritis. *Proc Soc exp Biol* (NY) 1970 *135* 384—388
GROSSMAN M I K K MATSUMOTO and R. J LICHTER Fecal blood loss produced by oral and intravenous administration of various salicylates *Gastroenterology* 1961 *40* 383—388
HOLSTI L. R T RASANEN and A CEDERBERG Epithelial mitoses and mucosal mast cells in the gastrointestinal tract of rat after whole body gamma irradiation and polysaccharides. Unpublished observation
JOHN D J B ST and F T MC DERMOTT Influence of achlorhydria on aspirin induced occult gastrointestinal blood loss Studies in Addisonian pernicious anaemia. *Brit Med J* 1970 ? 450—452
JOHNSON L R. Source of the histamine released during damage to the gastric mucosa by acetic acid *Gastroenterology* 1968 *54* 8—15
JOHNSON L J and B F OVERHOLT Release of histamine into gastric venous blood following injury by acetic or salicylic acid. *Gastroenterology* 1967 *52* 505—509
LEVITAN H and J L BARKER Membrane permeability Cation selectivity reversibly altered by salicylate *Science* 1972 *178* 63—64
OVERHOLT B F D A BRODIE and B J CHASE Effect of the vagus nerve and salicylate administration on the permeability characteristics of the rat gastric mucosal barrier *Gastroenterology* 1969 *56* 651—658
OVERHOLT B F and H M POLLARD Acetylsalicylic acid and ionic fluxes across the gastric mucosa of man *Gastroenterology* 1968 *54* 538—542
RASANEN T Tissue eosinophils and mast cells in the human stomach wall in normal and pathological conditions *Acta path microbiol scand* 1958 Suppl 1?9
RASANEN T Effects of dexamethasone prednisolone and cortisol on the mast cells and tissue eosinophils in rat gastric mucosa *Acta endocr* Kbh 1962 41 43 476
RASANEN T A mucosal bleeding mechanism in the upper part of the gastrointestinal tract. *Gastroenterology* 1963 *44* 168—1?
RASANEN T Some aspect of the humoral mechanism of gastric secretion In Gastric Secretion eds T K Shnitka J A L Gilbert and R C Harrison Pergamon Press 196? pp 55—261
RASANEN T The role of the mucosal cells in gastric ulceration In *Peptic Ulcer* Ed C J Pfeiffer Munksgaard 1971 pp 237—243
RASANEN T A CEDERBERG and E TASKINEN The mitotic count in the gastric intestinal epithelium and regenerating liver of heparinized rats *Gastroenterology* 1 ? 50 41—44
SHAPIRO S M H REDISH and H A CAMPBELL A study on prothrombin IV the prothrombinogenic effect of salicylate in man *Proc Soc exp Biol* NY 1943 53 251—254
SUGA ? C C E LUCAS and A J WALT Effect of histamine and aspirin on ? acute acetic acid ulcers *Mich Med* 1970 ?8
WINKELMAN E I and W H J SUMMERSKILL Gastric secretion in relation to gastrointestinal bleeding from salicylate compound *Gastroenterology* 1961 *40* 56—63

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Electrical Stimulation of Human Thalamic and Amygdaloid Area and Plasma Cortisol Concentration

By

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Abstract

LEPPALUOTO J *Electrical stimulation of human thalamic and amygdaloid area and plasma cortisol concentration* Acta physiol scand 1973 *89* 187—191

The concentration of plasma ortisol was measured from blood samples drawn through indwelled catheters at 3—5 min intervals in 4 resting healthy subjects and in 5 patients after electrical stimulation of the thalamus and amygdala via chronic electrodes implanted for the treatment of psychomotor seizures In the healthy subjects (labo atory staff) the pla ma cortisol level measured by a fluorometric method was initially high (10—37 μg/100 ml) evidently due to emotional affectivity of the experimental situation and decreased ith n 10 min by 10 μg in 2 subjects a d the increased within 10 min by 5—20 μg in 3 subjects These rapid changes of the plasma cortisol level bursts were significant and are a new physiological phenomenon In the patients the thalamus and amygdala were stimulated 30 s (pulse duration 1 ms repetetive frequency 50 Hz unidirectional square wave pulses) to *the voltage level* at which the patient *felt* a subjective sensation (*4—12 V*) After repetetive stimulations of thalamus (3 pat ents 6 times) and amygdala (2 patients 3 times) the changes in the plasma cortisol levels were inconsistent and sim lar to tho e seen in the healthy subjects No cor lusion about the function of the pituitary adrenal axis and amygdala or thalamus was drawn because the plasma cortisol values were evidently influenced by their temporal relat on to the secretion bu sts presented here

In human beings stereotaxic neurosurgical treatment offers a pos ibility to study the effects of electrical stimulation of CNS areas on neural and humoral function It has previously been found in one study that electrical stimulation of the limbic system modifies the function of the pituitar adrenocortical axis (Mandell *et al* 1962) These changes however resemble those found after repeated blood samplings in healthy subjects (Hellman *et al* 1970)

This paper is concerned with the concentrations of plasma cortisol after electrical stimulation of various CNS area and after repated blood samlings in human beings

Methods

Patients For the t eatment of underlying CNS disease unipol fle bl tainl ss w re electrodes (0 08 mm th ck) were mplanted tereotax ally in arou CNS s tes n 5 patients (Table I) b mean of a tereotaxic instrument (Laitinen 19 1) Se e al weeks later

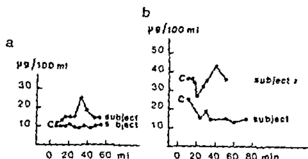


Fig 1 a and b Fluctuation of the plasma cortisol concentration as a function of time in 4 resting subjects C denotes the insertion of the catheter and points are means of 2 cortisol estimations Note the bursts in the plasma cortisol level in subjects 1 3 and 4

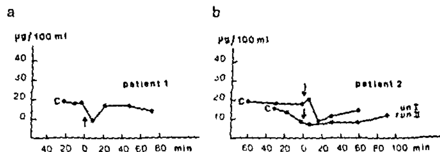


Fig 2 a and b Fluctation of plasma cortisol concentration as a function of time in 2 patient before (—) and after the electrical stimulation of amygdala (arrow) Se legend to Fig 1

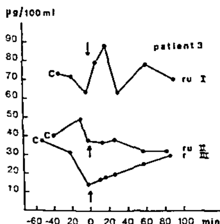
tients were transferred in bed to the operating theatre and a catheter inserted into the ...ital vein 40—60 min later a 30 s train of unidirectional square waves (50 Hz duration ms) was applied with a Grass stimulator and isolation unit (model S 4 and SIU B) The stimulus voltage was monitored and slowly raised on the threshold level (4 ... V) at which the patient felt a subjective sensation Blood was collected for 1 ... h and exteroceptive stimuli during that time were avoided Blood was collected only once from ... ambulant patie... without electrodes These experiments were carried out between 9 a m and ... p m

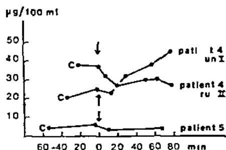
Healthy subjects In 4 volunteers (male laboratory staff no previous medical history aged 20—30 years) a catheter (70 cm long) was inserted into the cubital vein and the subject then lay on a bed in a sound shielded anechoic room for 1 h (Bergström *et al* 19..) ... was collected from the subjects by the catheter from outside the room between ... p m and 5 p m

Measurement of plasma concentration of cortisol Blood was taken into ... heparinized test tubes and centrifuged immediately The plasma cortisol level was determined (from ... samples) by the fluorescence method of Spencer Peet *et al* (1965) S 15 ... range of the plasma cortisol concentration was regarded as significant

Results

Healthy subjects At the beginning of the collection the plasma cortisol level varied from 10 to 37 µg/100 ml in 4 subjects (Fig 1 a and b) In subjects 3 and 4 the plasma cortisol level then decreased by about 10 µg/100 ml within 10–15 min Soon after this there was a rise in the hormone concentration ... µg/100 ml in subject 4 10 µg/100 ml in subject 1 and even 20 µg/100 ml in subject 3 who complained of feeling faint The plasma cortisol level was unchanged during the collection in subject 2 i e 10—8 µg/100 ml





Fi 3 a and b Fluctation of the pla ma cortisol concentration as a function of time in three patients before (—) and after the electrical stimulation of thalamus (arrow) Se legend to Fig 1

Electrical stimulation of the amygdala The plasma cortisol level fell from about 20 to 10 μg/100 ml in 10 min in 2 cases and then returned to the initial level When the stimulus was repeated about one month later no change could be seen (Fig 2 a and b)

Electrical stimulation of the thalamus Patient no 3 was stimulated 3 times The plasma cortisol level was initially high (37—73 μg/100 ml) although the patient was somnolent After electrical stimulation a rise a fall and a composite respon e in the plasma cortisol level could be seen (Fig 3 a) Patient 4 was stimulated twice

TABLE I

Patient no	Age years	Sex	Diagnosis	Approx mate electrode site	Medicat on
1	22	M	Tempo l l be epilepsy	Am ygdala	N l
2	27	M			Chlorpromaz n
3	19	F	*Myoclonus ep l ps*	*Thalamu*	*Carbama p ne* phen ob bital and d ph nylh dantoin
4	16	M			
5	66	F	Paralysis gitans		Perph naz n and cy l zine
6	24	F	M oclonus pil ps	No el ctrodes	Ca bamazep ne ph n ba b tal and d phenylh d nto n
7	26	F			

M = male F = f m le
The surg ry was carr ed out by L V Lait nen M D at the Neurosurg c l Cl n c H ls nki

The plasma cortisol level was initially somewhat high and presented a rise and a fall after stimulation (Fig 3 b) In 2 other patients who had a focus in the thalamus but not electrodes the plasma cortisol level was normal 10 and 13 μg/100 ml In patient 5 the plasma cortisol level was low and did not change after stimulation and lesioning of the ventral thalamus (Fig 3 b)

Discussion

In healthy subjects the plasma cortisol concentration in the morning is reported not to exceed 15 μg/100 ml by the method used (Spencer Peet *et al* 1965) In the present study higher plasma cortisol levels were occasionally found in the afternoon in 2 subjects and continually during the observation time in one subject The insertion of the catheter and the anticipation of the experiment seemed to be here emotionally affective and evidently elicited these high hormone levels The great emotional affectivity of the first experiment was later confirmed in this laboratory when the experiment was repeated with the same subjects the plasma cortisol was at a low and constant level (Laisi *et al* 1973) This is in agreement with previous findings in which raised cortisol secretion has been observed in corresponding first time experiments (Shabsin *et al* 1957)

The secretion of ACTH and cortisol has been previously shown to occur in sporadic episodes with a frequency 6—12 per day (Berson and Yalow 1968 Hellman *et al* 1970) In those studies the sampling interval was 20—60 min and hence the bursts with shorter duration could not be detected In the present study significant (about 70 % from the initial level) increases of plasma cortisol level bursts could be demonstrated within 5—10 min in healthy subjects This is a new physiological phenomenon as far as we know Its existence is supported by the following facts firstly the increases of plasma cortisol level exceeded the error of measurement method (15 %) secondly the changes in several samples near to a burst were progressing in the same direction and thirdly the half time of the decrease of plasma cortisol level did not exceed that previously seen in the first phase disappearence curve of cortisol in blood (Hellman *et al* 1970) From the present results it appears that one sampling every 3—5 min is sufficient to define the physiological events involved in cortisol secretion

In patients suffering from myoclonic and temporal lobe epilepsy the pre stimulation plasma cortisol concentrations were generally high but did not increase further after the insertion of the catheter (except patient 3 run II) In patient 5 the plasma cortisol level was low possibly owing to heavy medication (see Table I) It was remarkable that when the experiments were repeated the plasma cortisol levels were still high So the transfer of the patient to the operating theatre appeared to be stressful every time Moreover in the patients in spite of anticonvulsive medication the pituitary adrenal cortex axis may be sensitized The disease per se myoclonic epilepsy does not seem to provoke a high plasma cortisol level because we observed in two other myoclonic patients a normal plasma hormone level

After repeated electrical stimulations of thalamus and amygdala no consistent changes in the plasma cortisol concentrations were observed and the inconsistent changes resembled those seen in this study in the healthy subjects This result is at variance with a previous study in which repeated electrical stimulation of the amygdala and hippocampus consistently elicited the same change in the plasma cortisol level (Mandell *et al* 1962) The divergence may be explained by different electrode placements (Mandell *et al* 1962) or by different pulse repetition frequencies Kovacs *et al* 1965), but it is also equally possible that on or off coming secretion bursts of cortisol may mimic a decrease or increase in the plasma cortisol concentrations (Hellman *et al* 1970) Because of the rapid fluctuation of plasma cortisol concentrations no conclusion about the function of the pituitary adrenal cortex axis in relation to electrical stimulation of thalamus or amygdala was drawn

References

BERGSTROM M R R BLÅFIELD H FRUHSTORFER Y HYTONEN and T JAUHIAINEN A Sound Attenuating Electrically Shielded Chamber for Experiments in Sensory Physiology *Acta physiol scand* 1969 *75* 330—332

BERSON S and R YALOW Radioimmunoassay of ACTH in Plasma *J clin Invest* 1968 *47* 2725—2751

HELLMAN L F NAKADA J CURTI E WEITZMAN J KREAM H ROFFWARG S ELLMAN D FUKUSHIMA and T GALLAGHER Cortisol Is Secreted Episodically by Normal Man *J clin Endocr* 1970 *30* 411—422

LAITINEN L A New Stereoencephalotome *Zbl fur Neurochir* 1971 *32* 67—73

LAISI U S LEISTI J LEPPALUOTO J LUSTIO J PARTANEN and P VIRKKUNEN Vigilance Growth Hormone and Cortisol Secretion in Man *Acta physiol scand* 1973 *89* 192—197

KOVACS S A SANDOR Z VERTES and M VERTES The Effect of Stimulation of the Habenular Nucleus on Pituitary Thyroid Function *Acta physiol Acad Sci hung* 1966 *30* 39—45

MANDELL A L CHAPMAN R RAND and R WALTER Plasma Corticosteroids Changes in Concentration after Stimulation of Hippocampus and Amygdala *Science* 1962 *139* 1212

SABSHIN M D HAMBURG R GRINKER H PERSKY H BASOWITZ S KORCHIN and J CHEVALIER Significance of Preexperimental Studies in the Psychosomatic Laboratory *Arch Neurol Psychiat* (Chic) 1957 *78* 207—219

SHENKIN H The Effect of Pain on the Diurnal Pattern of Plasma Corticoid Levels *Neurology* (Minneap) 1964 *14* 1112—1117

SPENCER PEET J J DALY and V SMITH A Simple Method for Improving the Specificity of the Fluorimetric Determination of Adrenal Corticosteroids in Human Plasma *J Endocr* 1965 *31* 235—244

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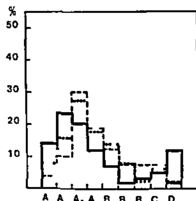


Fig 1 Percentage distributions of the EEG stages (Bente 1964) in different experiments Whole line denotes *experiment 1 (resting condition)* broken line expt 2 before the painful stimulus and dotted line expt 2 after the painful stimulus

Methods

In the first experiment surface electrodes were fixed to the head neck and wrists of the subject for monitoring EEG EMG and ECG For blood collection a catheter was inserted into the cubital vein Then the subject rested alone with closed eyes on a bed for 30 min in a sound shielded anechoic room (Bergstrom *et al* 1966) EEG EMG and ECG were monitored for 15 s every minute Blood (12 ml) was collected through the cath ter (volume 8 ml) from the room every 1—3 min

In the second experiment the procedures were similar except that faradic current was applied to the forearm for 10 s in the middle of the experiment The intensity of the current was raised just to the pain level The subjects were informed of the experiment 1—2 days earlier and were also given notice just before the application of stimulus

EEG stages were estimated according to the criteria used by Bente (1964) and Fruhstorfer and Bergstrom (1969) EMG peaks per minute and pulse rate were calculated Plasma cortisol concentration was measured by a micromodification of the fluorometric method of Spencer Peet *et al* (1965) Plasma growth hormone was determined by radioimmunoassay with carbon dextran separation of the bound and unbound hormone (Jacobs 1969) The standard used was NIH GH HS 1216 C received as a gift from Dr A. E Wilhelmi through National Institute of Arthritis and Metabolic Diseases Bethesda, Md U S A A 15 % change in the plasma growth hormone and cortisol level was regarded as significant The comparison between the variables was made by Student s *t* test

Results

Expt 1 EEG stages plasma growth hormone and cortisol concentrations in resting conditions

In this sound shielded anechoic environment vigilance of the test subjects fluctuated after about 10 min between real sleep and wakefulness Sleep stages represented 20 % and A stages represented 70 % of all the EEG stages (Fig 1) Pulse rates and EMG closely followed the EEG stages but showed great individual variation

The plasma growth hormone level was low (0—4 ng/ml) in 3 subjects but was unexpectedly high and variable in subject 2 It showed in this case three significant secretion burst during 20 min (Fig 2) The increases from the pre burst level were 130 37 and 56 %

The plasma cortisol level was at low and constant level (6—14 μg/100 ml) and no secretion episodes could be seen in any subject The plasma growth hormone level

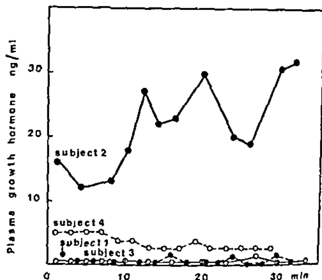


Fig 2 Fluctuation of the plasma growth hormone concentrations as a function of time in 4 resting subjects Note the 3 significant bursts at 12 20 and 30 min in subject 2

was generally high when vigilance was also high and then decreased with declining vigilance (Fig 3) The plasma cortisol level was however not affected by vigilance fluctuations (Fig 4)

Expt 2 EEG stages and plasma cortisol concentrations before and after application painful stimulus

The frequency distribution of the EEG stages before and after the stimulus were quite similar but there were less sleep stages (C and D) in expt 2 than in expt 1

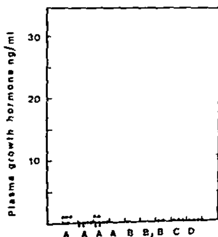


Fig 3

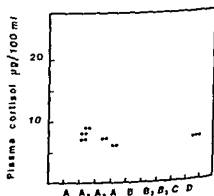


Fig 4

Fig. 3 Relation between the plasma growth hormone concentration and EEG stages in 4 resting subjects. Note that on stages B₁—D there are no values above the zero line

Fig 4 Relation between the plasma cortisol concentration and EEG stages in 4 resting subjects

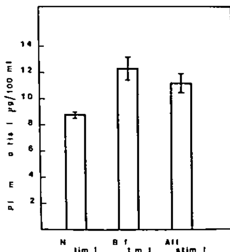


Fig 5

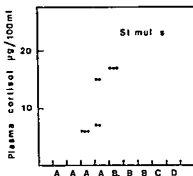


Fig 6

Fig 5 Plasma cortisol concentrations of 4 subjects in resting condition (No stimulus) before the application of faradic current (Before stimulus) and after the application of faradic current (After stimulus) Columns indicate means and bars denote S E

Fig 6 Relation between the plasma cortisol concentration and EEG stages in the experiment in which faradic current was applied (Stimulus)

(Fig 1) Just after the application of faradic current to the forearm vigilance rose sharply to A_1 stage for 1—2 min

The plasma cortisol level in expt 2 was initially higher and varied between individuals more than in expt 1 (5—18 μg/100 ml) The plasma cortisol concentration did not significantly change during 25 min after the painful stimulus

The average plasma cortisol level in expt 1 when no stimulus was applied was 8 9±1 5 μg/100 ml (mean±S D n = 67) It rose significantly ($p < 0\ 01$) in expt 2 in which a short painful stimulus was applied The average plasma cortisol level was 12 3±4 6 (n = 24) before the pain stimulus and 11 2±4 4 μg/100 ml (n = 37) after it The difference was not significant ($p > 0\ 05$) See Fig 5

The plasma cortisol level was not affected by vigilance fluctuations (Fig 6)

Discussion

In human beings plasma concentrations of cortisol ACTH LH and TSH has recently been shown to have 2 kinds of temporal fluctuations circadian rhythm with a frequency 1 per day (Berson and Yalow 1968 Vanhaelst *et al* 1972) and secretion episodes 6—14 per day (Berson and Yalow 1968 Hellman *et al* 1970 Boyar *et al* 1972 Vanhaelst *et al* 1972) In these studies the sampling interval has been 20—60 min which does not make it possible to detect rhythms with a frequency below 20—60 min The bursts and quiescent period in the secretion of some adenohypophyseal hormones and cortisol and the rapid disappearance of these hormones in blood (Hellman *et al* 1970 Boyar *et al* 1972) however allows the existence of significant secre

tion bursts with an estimated duration of 5—10 min In a previous study from this laboratory it was shown by frequent sampling blood sample every 2—4 min) that significant cortisol bursts occur with a duration of 5—10 min (Leppäluoto 197?)

In the present study the measurements of the plasma growth hormone levels demonstrated in an awake non fasted subject 3 significant secretion bursts within 20 min In the 3 other subjects the plasma growth hormone level was low even non detectable and the changes were not so distinct Hence growth hormone may also be secreted in bursts and the nature of this new physiological phenomenon will be established in a more extensive study (in preparation) It is noteworthy that these growth hormone bursts were not associated with the change in the plasma cortisol level.

In resting conditions the plasma cortisol level measured from the same samples as growth hormone was low and showed no secretion bursts up to 30 min We attempted to provoke secretion burst of cortisol by a short painful stimulus The post stimulus plasma cortisol level was however in all the subjects unchanged although more variable than in resting conditions (see S E in Fig 3) probably indicating the existence of nondetectable bursts It seems that the stimulus used here was too small because e g auditory stress for one hour (Arguelles et al 1965) or lumbosacral herniation (Shenkin 1958) for at least a day is required to increase the post stimulus plasma cortisol level Another phenomenon was seen here the pre stimulus plasma cortisol level was significantly increased when compared with the situation in which no stimulus was applied This is probably due to anticipatory mechanism because the subjects were aware of the painful stimulus before the experiment A raised resting level of urinary corticosteroid before an unfamiliar experiment has been similarly shown in a previous study (Sloane et al 1958)

In these short time experiments there was no correlation between vigilance and plasma cortisol level but there was a slight positive correlation between vigilance and plasma growth hormone level This indicates that a high vigilance level plasma growth hormone level This indicates that a high vigilance level plasma growth hormone concentration increases but decreases when vigilance is low The present finding of low plasma growth hormone concentration during sleep stages is at variance with the generally accepted concept of the surge of this hormone during sleep (Quabbe et al 1966 Lucke et al 1972 Mace et al 1972 The duration of sleep was in these experiments only 1–3 min and evidently insufficient to initiate a growth hormone burst The presence of a positive correlation between vigilance and plasma growth hormone concentration and the existence of hypothalamic control of growth hormone secretion suggest that the increases of vigilance could trigger the endocrine function of hypothalamus

References

Arguelles A E D Ibeas J P Ottone and M Chekherdemian [illegible] Adrenal S[illegible] lation by Sound of Different Frequencies J clin Endoc [illegible] PH [illegible]
Benze D, Die Insufflation der [illegible] aus Hab[illegible] Erlangen [illegible]
Bergstr[illegible] R M R Blåfield H Frühstorfer [illegible] and T J [illegible]

Attenuating Electrically Shielded Chamber for Experiments in Sensory Physiology *Acta physiol scand* 1969 *75* 330—332

BERSON S A and R. S YALOW Radioimmunoassay of ACTH in Plasma *J clin Invest* 1968 *47* 2725—2751

BOYAR R M PERLOW L HELLMAN S KAPEN and E WEITZMAN T ent Four Hour Pattern of Luteinizing Hormone Secretion in Normal Men with Sleep Stage Recording *J clin Endocr* 1972 *35* 73—81

FRUHSTORFER H and R M BERGSTROM Human vigilance and auditory evoked responses *Elect oenceph cl n Neurophysiol* 1969 *27* 346—355

HELLMAN L F NAKADA J CURTI E WEITZMAN J KREAM H ROFFWARG S ELLMAN D FUKUSHIMA and T GALLAGHER Cortisol Is Secreted Episodically by Normal Man *J clin Endocr* 1970 *30* 411—422

JACOBS H S Use of activated charcoal in radioimmunoassay of human growth hormone in plasma *J clin Path* 1970 *22* 710—717

LEPPALUOTO J Electrical stimulation of human thalamic and amygdaloid areas and plasma cortisol concentration *Acta physiol scand* 1973 *89* 187—191

LUCKE C N ADELMAN and S M GLICK The Effect of Elevated Free Fatty (FFA) on the Sleep-Induced Human Growth Hormone (hGH) Peak *J clin Endocr* 1972 *35* 407—41?

MACE J W R W GOTLIN and P BECK Sleep Related Human Growth Hormon (GH) Release A Test of Physiologic Growth Hormone Secretion in Children *J clin Endocr* 1972 *34* 339—341

QUABBE H J E SCHILLING and H HELGE Pattern of growth hormone secretion during a 24 hour fast in normal adults *J clin Endocr* 1966 *26* 1173—1177

SHENKIN H A The effect of pain on the diurnal pattern of plasma corticoid levels *Neurology* 1964 *14* 1112—1117

SLOANE R. B M SAFFRAN and R A CLEGHORN Autonomic and Adrenal Responsivity in Psychiatric Patients Effect of Methacholine and Corticotropin *Arch Neurol & Psychiat* (Chic) 1958 *79* 549—553

SPENCER PEET J J R DALY and V SMITH *A simple method for improving the specificity* of the fluorimetric determination of adrenal corticosteroids in human plasma. *J Endocrin* 1965 *31* 235—244

VANHAELST L E VAN CAUTER J P DEGAUTE and J GOLSTEIN Circadian variations of serum thyrotropin levels in man *J cl n Endocr* 1972 *35* 479—482

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From the Department of Physiology Veterinärhögskolan Stockholm Sweden

Secretory Fibres in the Trigeminal Part of the Lingual Nerve to the Mandibular Salivary Gland of the Rat

By

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Abstract

HELLEKANT G and Y KASAHARA *Secretory fibres in the trigeminal part of the lingual nerve to the mandibular salivary gland of the rat* Acta physiol scand 1973 89 198—207

The nerve supply to and the secretion from the mandibular salivary gland of the rat have been studied It was found that a) secretory fibres are given off from the chorda lingual nerve on its peripheral course until it reaches the mandibular and sublingual ducts b) secretion of saliva was observed and secretion could be evoked even when the facial portion of the chorda lingual nerves was cut The flow did not stop until its trigeminal portion was also cut c) the cessation of secretion after the trigeminal portion was cut was not the result of a loss of sensory impulses to the CNS d) impulses could also be recorded from the nerve fibres given off from the chorda lingual nerve to the gland after its facial portion was cut e) electrical stimulation of the peripheral part of the trigeminal portion elicited secretion which was generally larger than that in response to stimulation of the facial portion Simultaneous stimulation of both portions gave the largest secretion f) no difference between the sodium and potassium concentrations in saliva elicited by stimulation of the two nerve portions was observed Atropine blocked the secretion caused by stimulation of any of the nerves

The peripheral part of the lingual nerve is formed by junction of a trigeminal and a facial portion The facial portion is called the chorda tympani nerve It is usually held that it contributes all the efferent fibres which can be found in the lingual nerve while the trigeminal portion is regarded as exclusively sensory (Vallbo er 1964 Thus it is stated in textbooks (*e g* Handbuch der Neurologie 1935 that *all secretory* fibres in the lingual nerve going to the mandibular salivary gland originate *from* the chorda tympani nerve

In an earlier study Hellekant 1971) recordings of central parts of gland branches from the chorda lingual nerve were made It was then observed that not all of the impulses that could be recorded emanated from its facial portion because a number of these impulses disappeared when its trigeminal portion was cut (Hellekant 1971 Fig 3) In view of our knowledge about this nerve outlined above it seemed

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that this observation deserved a further investigation Therefore this study was made It will show that a considerable number of the secretory fibres to the mandibular salivary gland in the rat emanate from the trigeminal portion of the lingual nerve and that only a part of the lingual secretory fibres emanates from the chorda tympani nerve

Methods

Male Sprague Dawley rats weighing between 450 and 550 g had been used They were anesthetized with Hypnorm® Hypnorm is a combination of a narcotic analgesic, 0 2 mg/ml of fenthanyl citrate and a tranquillizer 10 mg/ml of fluanison especially adapted for veterinary use The initial dose of 0 5 ml/kg b wt was injected i m Additional small doses were then injected when necessary In some animals small doses of pentobarbitone not more than 10 mg/kg b wt were added during the dissection This was done when the muscular activity of the animal seriously disturbed the dissection. Some animals were artificially ventilated after i v injection with gallamine

One femoral vein and one femoral artery were always cannulated to allow i v injections and recording blood pressure The rectal temperature was maintained at 37±1 C The right mandibular salivary duct was cannulated with polyethylene tubing with internal and external diameters of 0 28 and 0 68 mm respectively The point of inserting the tubing was always orally to the intersection between the chorda lingual nerve and the salivary ducts When the tubing had been inserted into the mandibular duct the passage was tested by an i v injection of about 50 μg pg pilocarpine/kg b wt The salivation caused lasted for about 45 min during which time the remaining dissections were done

The rate of flow of saliva was determined in 3 ways. The free opening of the tubing was observed with a microspoce As soon as a drop had reached a certain size it was absorbed with a piece of cotton and a signal was given to a recorder The second method was used when the latency was determined between the onset of stimulation of either the chorda tympani proper or the lingual nerve proper and the first drop of saliva elicited. Then an electrode connected to an amplifier was placed above the opening of the cannula. When a drop had reached a certain size it made contact with the electrode This gave an artefact which was recorded The third method was used when the flow rate was too high so that the number of drops could not be counted satisfactorily Then the increase in weight of a cotton wool bud due to the absorption of saliva during 30 to 60 s was used Saliva was also collected for sodium and potassium determinations The chorda tympani proper and the lingual nerve proper were alternatively stimulated for about 2 to 3 min while saliva was collected in small test tubes The stimulation applied was adjusted to give about the same flow in all experiments The determinations of Na and K were made on a flame photometer 5 animals were used and 20 samples were collected

The nerve supply to the mandibular salivary gland is shown schematically in Fig 1 The nomenclature used is based on a description by Langley (1898) The right chorda tympani proper and the lingual nerve proper were dissected but not cut In some cases some of the nerve filaments which constitute the chorda tympani were dissected for recording The recordings from the nerves and the stimulations of the nerves were made under mineral oil The nerve impulses were amplified with a PAR 113 amplifier and displayed on a storage oscilloscope Tektronix 5103N The impulses could also be recorded on a Stratos I writer Varian Assoc Mechanical stimulation of the tongue was done manually with a brush Grass S48 and S4 stimulators were used for electrical stimulation

Histological studies were made on some animals The nerves and the ducts were either fixed in Bouin's solution and stained in hematoxylin-eosin or fixed and stained in thionin (Ehrlich) and then mounted in paraffin The tissue was cut in transverse sections 10 μm thick and examined under the light microscope

Results

Anatomical observations

Fig 1 above gives a schematic picture of our anatomical findings The chorda lingual nerve on its peripheral course gives off branches in the direction of the salivary glands These bundles constitute the chorda tympani nerve Their number differed

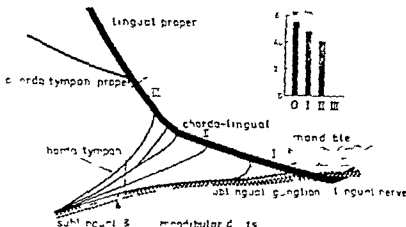


Fig. 1. A generalized diagram of the anatomy of the nerve supply to the mandibular and sublingual salivary glands. The nomenclature is adopted from Langley (1898). The effect of section of the chorda lingual nerve at different levels on the secretion evoked by electrical stimulation of the lingual proper at 5 cps in one animal is also shown. The roman numerals indicate the order and position of the sections made. The secretion obtained after each cut is shown in the inset diagram. The left hand column (0) was obtained before sectioning.

from animal to animal. Some of them were so small that it was difficult to identify them under the dissection microscope. They were ensheathed with supporting tissue and they converged as they approached the salivary glands. Ganglion cells were intermingled with the bundles. The ganglion cells were most abundant close to the point of contact with the ducts. These scattered cells form probably the sublingual ganglion described in other mammalian species (Langley 1898) but in which it seems to be better defined. The parts of the ducts which were accessible without disturbing the nerve supply to the glands were a few millimetres long. They were situated between the intersection with the chorda lingual nerve and the edge of the mandible (see Fig. 1). Our serial sections show that some of the bundles given off do not contain any fibres from the chorda tympani proper. It might be thought that these bundles consisted of sensory fibres, but as will be shown below, we found that they contained secretory fibres too.

Observations during mechanical stimulation of the tongue

It is well known that mechanical stimulation of the tongue can elicit reflex salivation. This was also found in our experiments. In this reflex the lingual nerve has been regarded as the sensory link with the chorda tympani proper mediating the efferent link. But our experiments showed that salivation could be evoked in animals where the chorda tympani proper had been cut. An example of this is shown in Fig. 2. The left and right columns in each group show the salivation rate before and after brushing the tongue. The central column shows the flow while the tongue was brushed. The results in A were obtained before the chorda tympani proper was cut, B after it had been cut, C after ipsilateral sympathectomy and D after the lingual nerve proper

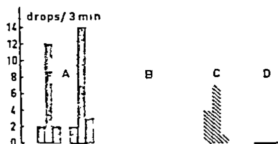


Fig 2 The effect of successive denervation on the secretion of the mandibular gland The left and right columns in each group show secretion before and after brushing the tongue The central column was obtained during brushing A was obtained with all nerves uncut B after the cutting the chorda tympani proper C after ipsilateral sympathectomy and D after the lingual nerve proper had been cut.

had been cut Fig 2 shows that salivation did not stop until the lingual nerve proper had been cut Later as a control pilocarpine was injected It elicited a strong salivation This shows that constriction or blockage of the duct or the tubing were not the causes of the cessation of salivary flow shown in D

Observations in other animals gave the same results without exception That is if secretion of saliva was observed and salivation could be evoked these effects were not abolished when the chorda tympani proper was cut but disappeared first when the lingual nerve proper was also cut

It is known that an uninterrupted sensory inflow from the oral region is necessary to maintain salivation It could be surmised that after cutting of the chorda tympani proper secretion was maintained through some unknown route and that the cessation described *after cutting of the lingual nerve proper* was due to a loss of sensory inflow Fig 3 shows that this explanation is not correct In this experiment the chorda tympani proper had been cut before the experiment was started In spite of this a flow of saliva was maintained at a constant rate A small dose of Xylocaine® was then applied to the tissue that contained the chorda tympani nerve bundles Care was taken not to expose the lingual nerve proper or the chorda lingual nerve to Xylocaine Fig 3 shows that the first application somewhat diminished the flow of saliva and that the second application abolished both this flow and the flow that could

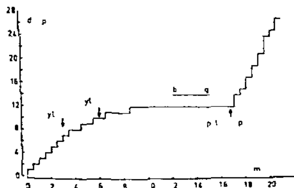


Fig 3 The diagram shows the secretion counted as drops plotted against time in a rat whose chorda tympani proper previously had been cut During this secretion Xylocaine was applied to the chorda tympani nerve The diagram shows that the anesthetic first impaired and then abolished the secretion The results show that the nerve fibres which maintain secretion after cutting of the chorda tympani proper emanate from the chorda lingual nerve

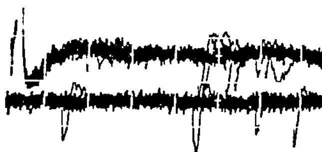


Fig 4 The records show the efferent impulse activity in a fine peripherally cut strand of the lingual proper nerve that otherwise was uncut. Each trace is composed of 10 superposed sweeps The upper one was obtained during mechanical stimulation of the tongue and shows a reflex response about 9 ms after the stimulation The lower trace was obtained when no stimulation was applied. Time scale 2 ms/div

previously be evoked by brushing of the tongue (not shown) The effect of an injection of pilocarpine showed that the gland was functional Later not shown in Fig 3 after several rinses with Ringer's solution an increase of salivatory rate in response to brushing of the tongue could again be recorded The results shown in Fig 3 demonstrate that loss of the sensory link is not the explanation of the cessation of salivation after cutting the lingual nerve proper because the sensory link from the mouth was left intact in this experiment Further the results show that the nerve fibres which maintained salivation after cutting of the chorda tympani proper emanated from the chorda lingual nerve

Observations during recording of nerve activity

The number of efferent fibres in the lingual proper is small compared to that of the sensory fibres In general this frustrated the attempts to record efferent impulses from this nerve However in one animal the lingual proper on one side consisted of two bundles one of about the size of the chorda tympani proper This was cut at its junction with the chorda tympani proper and recordings were made from its central part The number of efferent impulses in this strand was about 20/s when no stimulation was applied Mechanical stimulation of the tongue gave a reflex increase of the activity This can be seen in the upper record of Fig 4 Fig 4 is composed of 10 superposed sweeps with a sweep speed of 2 msec per vertical line The mechanical

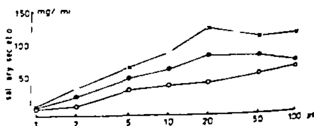


Fig 5 The flow of saliva evoked by electrical stimulation of the chorda tympani proper the lingual nerve proper and both nerves at different stimulation frequencies has been plotted against the frequencies applied The results show that most secretion was obtained when both nerves were stimulated and that the flow evoked by stimulation of the lingual proper exceeded that evoked by chorda tympani proper stimulation. Symbols O = chorda tympani proper ● = lingual proper × = chorda tympani proper and lingual proper

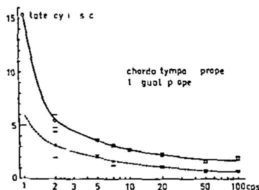


Fig 6 The lantency between onset of electrical stimulation and secretion caused has been plotted against the frequency applied The symbols represent the average of three determinations in the same animal The range is shown by the small horizontal lines

stimulation was applied to the tongue at 10 Hz and gave as shown by Fig 4 a reflex response after about 9 msec The lower trace shows the activity in the nerve during the same time when no stimulation was applied Four impulses were recorded The above shows that there are efferent fibres in the lingual proper and that mechanical stimulation of the tongue gave a reflex response

Recordings were also obtained from the nerve bundles which constitute the chorda tympani nerve before and after cutting of the chorda tympani proper The results obtained show that some of the nerve bundles which branched from the lingual portion of the chorda lingual nerve contained efferent fibres which originated from the lingual proper because the impulses remained when the chorda tympani proper had been cut

Observations during electrical stimulation

Electrical stimulation of the peripheral end of the lingual nerve proper as well as of the chorda tympani proper readily elicited secretion from the mandibular gland This was shown in all of 15 animals tested Electrical stimulation of the lingual nerve proper gave a larger secretion than stimulation of the chorda tympani proper in all animals except two This was observed at all frequencies used Fig 5 shows an example of this Two stimulators were used Each nerve was connected to its own stimulator Four volts of 1 msec duration was applied to each nerve Each stimulation frequency was applied first to the two nerves simultaneously then to each separately and finally to both nerves again This last stimulation served as a control that no

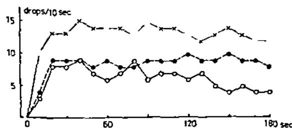


Fig 7 The flow of saliva evoked by electrical stimulation 3 cps has been plotted against time in one animal to show the effect of extended stimulation The results indicate that the flow caused by lingual proper stimulation diminished less than that due to chorda tympani proper stimulation Symbols as in Fig 5

fatigue of the gland or changes in stimulation condition had interfered The stimulation period lasted for 1 min when frequencies of 5 Hz or below were used and for half a min to higher frequencies This was done in order to diminish the possible effect of fatigue of the gland The order between the stimulus frequencies was randomized Fig 5 shows that at all frequencies applied stimulation of the lingual proper caused more salivation than did stimulation of the chorda tympani proper Further it shows that simultaneous stimulation of the two nerves gave the highest flow though this flow was not as large as the sum of the separate flows No exception to this last rule was observed In four animals constant current stimulation was applied to the nerves instead of constant voltage In all four animals more saliva was elicited to stimulation of the lingual proper than to stimulation of the chorda tympani proper

The latency between onset of stimulation and the onset of salivation differed for the two nerves It was shorter for the flow caused by stimulation of the lingual proper An example of this is shown in Fig 6 In this experiment the method of flow determination described was used Each stimulus frequency was repeated three times and the average value was calculated These values were then plotted against the frequency used The small horizontal lines in Fig 6 illustrate the range The curves have been fitted by eye Fig 6 shows that the interval between stimulation and salivation was about twice as long at all frequencies used when the chorda tympani proper was stimulated as when the lingual proper was Provided that supra maximum stimulation was applied there are two possible explanations for this First more secretory cells are innervated by fibres from the lingual proper than by those of the chorda proper Second the nerves may innervate different kinds of cells within the gland which contains (Babkin 1950) not only secretory cells but also myoepithelial cells These cells have been assumed to be contractile (Emmelin Garrett and Ohlin 1968 1969) which may explain the short latency To test this second possibility we stimulated the two nerves for longer periods to see if there was a difference in the diminution of flow rate with time The results of such an experiment are plotted in Fig 7 Four volts 3 Hz was used while the number of drops of saliva over 10 s was counted The result was then plotted against time of stimulation Fig 7 shows that the flow caused by stimulation of the lingual proper diminished less than that caused by chorda tympani proper stimulation Thus there is no indication that the fibres in the lingual proper do not innervate secretory cells As a comparison the result of stimulating the two nerves simultaneously in the same experiment has been added It shows as in Fig 4 that stimulation of both nerves gave a much larger flow than stimulation of either alone Both flows were blocked by atropine 0 5 mg kg b wt

It was mentioned earlier that nerve bundles are given off from the chorda lingual nerve in the direction of the mandibular salivary gland These bundles contain secretory fibres as was shown in the following experiment The chorda lingual nerve was successively cut at the three different levels indicated by the Roman figures (I—III) in Fig 1 The first cut was made at I The distance between each cut was

TABLE I. Sodium and potassium concentration in salivary samples obtained during stimulation of the lingual proper and chorda tympani proper

Nerve stimulated	Na mEq/l $\bar{x}$ S.D.	Number of samples n	K mEq/l $\bar{x}$ S.D.	Number of samples n	Flow µl/mg/min $\bar{x}$
Lingual proper	5.1±2.0	10	33.0±4.2	10	0.13
Chorda t. proper	5.6±2.0	10	34.1±3.9	10	0.13

about 3 mm. After each cut the lingual nerve proper was electrically stimulated with 4 V, 3 Hz. The result obtained is shown in the diagram in the right hand corner of Fig. 1. The left hand column was obtained before any cut was made. The experiment was repeated in 4 rats with similar results. This, together with the results obtained with histological technique and during the recordings, shows that secretory fibres are given off from the chorda lingual nerve on a length of about 10 mm before it reaches the salivary ducts.

Observations of the sodium and potassium concentrations in saliva

It is known that the concentrations of these electrolytes in the saliva are influenced by the type of nerve which gives rise to the secretion. Therefore it seemed of interest to study the possible difference in saliva caused by lingual proper and chorda tympani proper stimulation. It is further known that the concentrations of these electrolytes are influenced by the flow rate because absorption and excretion occurs in the ducts. We therefore adjusted the stimulus rate before we collected a sample to elicit the same flow rate in each experiment. The samples collected were also weighed. The results obtained are shown in Table I. (The average values for each ion is listed with its S.D., number of samples from which it was calculated and the average flow rate observed during each nerve stimulation.) The figures obtained indicate that there were no differences with regard to the sodium and potassium contents in the two types of saliva.

Discussion

One component of the analgesic used in this study, fenthanyl citrate, is a derivate of morphine which is known to cause salivation (Emmelin 1953). This may explain the sustained salivation after the chorda tympani proper was cut. However, it does not explain the cessation of salivation after section of the lingual proper, nor the salivation caused by electrical stimulation of this nerve, nor the increase caused by mechanical stimulation of the tongue. With regard to this last observation we want to emphasize that we were not able to elicit salivation by brushing the tongue in all animals. We even observed in one rat with a good flow of saliva that the flow diminished and disappeared during brushing of the tongue. It did not return until we stopped the brushing. But this was the case also before we had cut the chorda tympani proper. Further, it is well known that the salivatory reflex is strongly modified by the CNS (Babkin 1950).

Earlier studies have demonstrated the existence of myoepithelial cells in the mandibular gland These cells seem to be contractile and able to expel saliva already secreted into the intraglandular ducts from the gland (Emmelin Garett and Ohlin 1968 1969) It may be suggested that the secretion caused by stimulation of the lingual proper was the result of the contraction of these cells and not a true secretion However a flow of saliva elicited along these lines would deteriorate more rapidly than a true secretion This was not the case as was especially observed during the electrolyte determinations which necessitated extended and repeated stimulation of the two nerves Further the fact that stimulation of the lingual proper in most animals gave a larger flow of saliva than stimulation of the chorda proper and that simultaneous stimulation of the two nerves in all animals gave the largest flow indicates that the secretion during lingual proper stimulation was not caused by any spread of current to the chorda tympani proper since it is unreasonable that indirect stimulation of a nerve should elicit larger effects than direct stimulation In some animals a string of silk which previously had been loosely tied around the lingual proper between the chorda lingual nerve and the electrodes was tightened Then no salivation could be elicited by stimulation of the lingual proper while the flow to chorda tympani proper stimulation was unaffected We therefore conclude that the lingual proper contributes secretory fibres in the rat

Our observations on the Na and K contents during the chorda tympani proper stimulaiton gave about the same concentrations of the electrolytes as found earlier (cf Schneyer and Schneyer 1972) Further we found no difference between the saliva caused by chorda tympani proper stimulation and that by lingual proper stimulation This indicates that the influence of these two nerves on the secretion of the gland was essentially the same Since the values obtained here were the same as found earlier during parasympathetic stimulation and the secretion to stimulation of both nerves was blocked by atropine it can be concluded that the secretory fibres in the lingual nerve proper probably are parasympathetic This is of interest in speculation about the origin of these fibres

At the moment we can think of two possibilities One is that the salivary nuclei extend forward to the origin of the Vth nerve It is also possible that the lingual proper receives same fibres from the glossopharyngeal nerve though such a contribution has to our knowledge not been suggested or demonstrated But the course of the secretory nerve supply to other glands for example the parotid gland has been shown to be more complex than earlier thought (Holmberg 1972)

There are data which indicate an unknown path for secretory fibres to the mandibular gland Laage Hellman and Stromblad (1960) concluded from observations in three patients who had their chorda tympani proper resected during middle ear surgery that there was an unknown parasympathetic pathway to this gland Similarly other authors (Herrmann 1965 Wiberg 1971) demonstrated secretion and a secretory response to acid from this gland even though all presumed parasympathetic fibres the chorda tympani proper had been cut and the sympathetic nerves blocked or demonstrated to have little effect Peronace et al (1961) presented data

obtained in denervation experiments in rat Their figures show a significant difference between the results obtained after cutting the chorda tympani proper and these obtained after cutting the chorda tympani However their technique of sectioning the chorda tympani involved cutting some blood vessels and therefore the interpretation of their results is difficult

Data which do not corroborate the results presented here have to our knowledge been obtained only by Stricker and Hainsworth (1970) They studied the time of survival during hyperthermia of 2 groups of rats One had their mandibular and sublingual glands removed In the other group the chorda tympani proper had been sectioned In both groups the parotid ducts were ligated Stricker and Hainsworth (1970) found no difference in survival time between the two groups Their results indicate that no secretory fibres are present in the lingual proper because otherwise rats with intact glands but sectioned chorda tympani proper would have been able to salivate more thereby surviving longer than rats without glands On the other hand there are minor sublingual glands which probably were left intact in the desalivation experiment and therefore able to secrete at a maximum rate while their ability to secrete as well as that of the major sublingual and mandibular glands was impaired in the denervation experiment

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References

BABKIN B P *Secretory mechanism of the digestive glands* New York Hoeber 2nd edn 1950 pp 1—1027

EMMELIN N On spontaneous secretion of saliva. *Acta physiol scand* 1953 *30* Suppl 111 34—58

EMMELIN N J R GARRETT and P OHLIN Neural control of salivary myoepithelial cells *J Physiol* (Lond) 1968 *196* 381—396

EMMELIN N J R GARRETT and P OHLIN Motor nerves of salivary myoepithelial cells in dogs *J Physiol* (Lond) 1969 *200* 539—546

GREVING-SCHWEINFURT R Makroskopische Anatomie und Histologie des vegetativen Nerven systems *Handb Neurol* Berlin Springer 1935 *I* 811—886

HELLEKANT G Efferent impulses in the chorda tympani nerve of the rat *Acta physiol scand* 1971 *83* 203—209

HERRMANN R Die funktionellen Folgen des Chorda tympani Ausfalles und ihre klinische Bedeutung *Z Laryng Rhinol* 1965 *44* 414—417

HOLMBERG J On the nerves to the parotid gland *Oral physiol* Pergamon Press 1972 1—3

LAAGE HELLMAN J E and C R STROMBLAD Secretion from human submaxillary gland after section of the chorda tympani *J appl Physiol* 1960 *15* 295—297

LANGLEY J N The salivary glands *Textbook of Physiol* Edinburgh & London Young J Pentland 1898 *I* 475—530

PERONACE A A V T A DAVISON A B HOUSSAY and C J PEREC Alterations in submandibular and retrolingual glands following parasympathetic denervation in rats *Anat Rec* 1964 *150* 25—34

SCHNEYER L H and C A SCHNEYER Responses of perfused main duct of rat submaxillary gland to pharmacological agents *Oral Physiol* 1972 pp 61—2

STRICKER E M and F R HAINSWORTH Evaporative cooling in the rat Effects of hypothalamic lesions and chorda tympani damage *Can J Physiol Pharmacol* 1970 *48* 11—17

VILLIGER E *Die periphere Innervation* 1964 12th edn 219 pp Schwabe & Co Verlag Basel/Stuttgart

WIBERG A *Function of the chorda tympani before and after operation for clinical otosclerosis* Dissertation 1971 pp 1—79 Umeå

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From the Department of Physiology Veterinärhögskolan Stockholm Sweden

Salivatory Fibres in the Trigeminal Part of the Lingual Nerve to the Mandibular Salivary Gland of the Monkey

By

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Abstract

HELLEKANT G and Y KASAHARA. *Salivatory fibres in the trigeminal part of the lingual nerve to the mandibular salivary gland of the monkey* Acta physiol scand 1973 89 208—212

In the monkey electrical stimulation at different frequencies was applied to the peripheral end of the centrally cut trigeminal and facial branches of the chorda lingual nerve while the secretion from the mandibular salivary gland was studied It was found that a secretion could be elicited from the gland by stimulation of either of the two branches but the secretion elicited by stimulation of the trigeminal branch was slightly smaller than elicited by the facial branch which is said to be the sole source of secretory fibres to this gland in the nerve [illegible] the sodium potassium contents of the saliva elicited by the two nerve branches did not differ and were similar to that observed in human saliva.

One branch of the trigeminal nerve the lingual proper receives in its peripheral course a branch from the facial nerve the chorda tympani proper which contributes the secretory fibres to the mandibular gland An earlier study Hellekant and Kasahara 1973 *describes observations obtained in the rat which* suggest secretory fibres also from the lingual proper to this gland Since the available literature states that all the secretory fibres in the chorda lingual nerve emanate from the chorda tympani proper Greving Schweinfurt 1935 Villiger 1964 this new pathway is of interest However its value is small if limited to the rat but will gain if demonstrated in a species more closely related to man

Methods

Two female monkeys *Cercopithecus aethiops* weighing [illegible] and [illegible] kg were used They were anesthetized with Mebumal® 1 ml of which contains 60 mg of pentobarbital sodium The initial dose of 0.5 ml/kg b wt was injected i p Additional small doses were then injected i v when necessary

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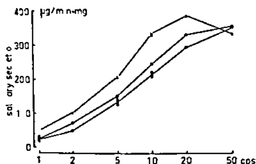


Fig 1

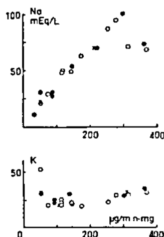


Fig 2

Fig 1 The flow of saliva evoked by electrical stimulation of the peripheral parts of the cut chorda tympani proper the lingual proper and of both nerves at different stimulation frequencies has been plotted against the frequencies applied The results show that most secretion was obtained when both nerves were stimulated simultaneously and that the flow evoked by stimulation of the lingual proper was almost as great as that evoked by chorda tympani proper stimulation Symbols ● = lingual proper ○ = chorda tympani proper × = chorda tympani proper and lingual proper

Fig 2 Relationship between concentration of sodium and potassium and rate of salivary flow obtained during stimulation of the chorda tympani proper (○) the lingual proper (●) and both nerves at the same time (×) Values from both monkeys are included The diagram indicates no difference in ion concentration which can be related to the type of nerve stimulated

The right mandibular salivary duct was cannulated with polyethylene tubing with internal and external diameters of 0 40 and 0 80 mm respectively The tubing was inserted orally to the intersection between the chorda lingual nerve and the salivary duct The rate of flow of saliva was determined by absorbing the saliva in a cotton wool bud over a certain time period and then measuring its increase in weight. A few samples were collected in small test tubes which were weighed The sodium and potassium content of the saliva was determined on a flame photometer After the experiments the mandibular salivary glands of both sides were dissected and weighed In one monkey stain was injected into the gland through the salivary duct as a control

The facial portion of the chorda lingual nerve the chorda tympani proper and the trigeminal portion the lingual proper were dissected and cut centrally Each nerve was put on two electrodes. Each pair of electrodes was connected to the stimulus isolation units of two Grass stimulators S48 and S4 Square wave pulses at different frequencies and a voltage of 3 V and a duration of 3 ms were used as stimulus The frequencies were applied in random order

Results

In both monkeys secretion from the mandibular salivary glands could be elicited with equal ease by stimulating the lingual proper and by stimulating the chorda tympani proper However in both animals the flow of saliva was somewhat smaller to lingual proper stimulation than to chorda tympani proper stimulation

Fig 1 shows the results obtained in one animal The flow obtained in response to each stimulation frequency has been plotted against the frequency used Stimula

tion lasted for 1 min for 1 and 2 Hz for 30 s for 5 and 10 Hz and for 15 s when 20 and 50 Hz were used This was done to fatigue the gland as little as possible Some of the stimulations were repeated to test this possible source of error This is indicated by more than one symbol at the same frequency in Fig 1 This figure shows that secretion was elicited by stimulation of the lingual proper (filled circles) Further it indicates that stimulation of the chorda tympani proper gave a slightly larger flow (open circles) than stimulation of the lingual proper Finally it illustrates that simultaneous stimulation of both nerves (crosses) gave the largest flow though this flow was less than the sum of the two separate flows

After the experiment a string of silk which previously had been loosely tied around the lingual proper between the chorda lingual nerve and the electrodes was tightened. Then no salivation could be elicited by stimulation of the lingual proper while the flow to chorda tympani proper stimulation was unaffected This suggests that the lingual proper in the monkey contributes secretory fibres to the mandibular salivary gland.

In Fig 1 stimulation periods of 1 min or less were used Observations during more extended periods of stimulation 2—3 min or during stimulations repeated at fairly short intervals did not reveal any differences between the two flows that could be related to the time This indicates that both nerves innervated secretory cells

It is known that the concentrations of sodium and potassium are influenced by the type of nerve which gives rise to the secretion (Emmelin 1971) It seemed of interest to study the concentration of these ions in saliva elicited by stimulation of these two nerves the chorda tympani proper and the lingual proper The concentrations of these ions in saliva elicited by stimulation of both nerves at the same time were also measured It is also known that the concentrations of sodium and potassium in saliva are influenced by the flow rate since considerable absorption and secretion takes place in the ducts Thus the final concentrations of these ions are related to the flow rate Fig 2 shows the results The contents of sodium upper curve and potassium lower curve have been plotted against the flow rate of saliva expressed in μg/min and mg weight of gland Values from both animals are included. The symbols used are the same as in Fig 1 It can be concluded from the data of Fig 2 that no difference in concentrations of these two ions that could be related to the type of nerve stimulated was observed Further it shows that the sodium content of the saliva increased with the flow rate and that initially there was a tendency for the potassium concentration to decrease with increased flow

Discussion

We have earlier described observations which indicate secretory fibres in the trigeminal portion of the chorda lingual nerve of the rat (Hellekant and Kasahara 1973) The number of rats in which we have obtained salivation from the mandibular salivary gland as the result of stimulation of this nerve now exceeds 50 In m

animal we have obtained negative results Therefore we think that the existence of these fibres in the rat is firmly supported

However we did not know if similar observations could be obtained in another species The experiments described here showed that salivation as the result of electrical stimulation of the lingual proper was easy to evoke in the monkey Further our observations both within each animal as well as between them were very consistent We therefore think that these results together with these referred to above (Hellekant and Kasahara 1973) enable us to suggest that the trigeminal portion of the chorda lingual nerve contains secretory fibres to the mandibular salivary gland in the monkey

This conclusion may seem premature, based as it is essentially on two animals but the experiments described here serve only to establish the existence or non-existence of these nerves In experiment of the present type one animal may be enough to allow a conclusion on the existence of these efferent fibres

The monkey was chosen because it is more closely related to man than rat is Most data which suggest an unknown pathway for secretory fibres to the mandibular gland have been obtained in man Thus Laage Hellman and Stromblad (1960) concluded that there is an unknown parasympathetic pathway to this gland Similarly other authors (Herrmann 1965 Wiberg 1971) demonstrated secretion and secretory response to acid from this gland even though all presumed parasympathetic fibres had been cut and the sympathetic nerves blocked or demonstrated to have little effect From the results of the present study we are inclined to suggest that there is a similar secretory pathway in man to that suggested here in monkey and that the observations described by these authors were the results of activity mediated by this pathway

In Fig 1 the stimulus periods were shorter when higher stimulus frequencies were applied This was done because we wanted to fatigue the gland as little as possible The fact that almost the same values were obtained when a stimulus was repeated indicates that the gland did not fatique However the curves of Fig 1 would probably be somewhat steeper if the same stimulus periods had been used

Fig 1 shows that the flow evoked by lingual proper stimulation was smaller than that by chorda tympani proper stimulation This was observed in both monkeys and may indicate that the proportion of secretory fibres in the two nerves differs from that in the rat which generally shows a larger secretion to lingual proper stimulation (Hellekant and Kasahara 1973) Further Fig 1 shows that the largest secretion was elicited when both nerves were stimulated but that this flow was less than the sum of the two separate This is in agreement with the observations in rat

Fig 2 shows no difference in the contents of sodium and potassium in the saliva that could be related to the type of nerve stimulated A similar lack of difference has earlier been observed in the rat (Hellekant and Kasahara 1973) This together with our observation in rat that atropine blocks both types of salivary flow indicates that the secretory fibres in the lingual proper are parasympathetic In addition Fig 2 presents values on the concentration of these ions in saliva of the monkey This has to our knowledge (Altman and Dittmer 1961 Schneyer and Schneyer 1967) not

been measured before The curves in Fig 2 are similar to those published by Thaysen Thorn and Schwartz (1954) for human parotid saliva and resemble those obtained for mandibular saliva in dog (Gregersen and Ingalls 1931)

References

ALTMAN P L and D S DITTMER, Blood and other body fluids *Fed Amer Soct exp Biol* 1961 *1* 399

EMMELIN N Control of salivary glands *Oral physiol* Pergamon Press 1972 *1* 1—16

GREGERSEN M I and E N INGALLS The influence of rate of secretion on the concentrations of potassium and sodium in dog s submaxillary saliva *Amer J Physiol* 1931 *98* 441—446

GREVING-SCHWEINFURT R Makroskopische Anatomie und Histologie des vegetativen Nervensystems *Handbuch d r Neurologie* I Ed Bumk and Foerster Springer Verlag Berlin 1935 pp 811—886

HELLEKANT G and Y KASAHARA, Secretory fibres in the trigeminal part of the lingual nerve to the mandibular gland of the rat. *Acta physiol scand* 1973 *89* 198—207

HERRMANN R Die funktionellen Folgen des Chorda tympani Ausfalles und ihre klinische Bedeutung *Z Laryng Rhinol* 1965 *44* 414—417

LAAGE HELLMAN J E and C R STRÖMBLAD Secretion from human submaxillary gland after section of the chorda tympani *J appl Physiol* 1960 *15* 295—297

SCHNEYER, L H and C A SCHNEYER Inorganic composition of saliva *Handbook of Physiol* Sec 6 1967 *2* 497—530

THAYSEN J H N A THORN and I L SCHWARTZ Excretion of sodium potassium, chloride and carbon dioxide in human parotid saliva *Amer J Physiol* 1954 *178* 155—159

VILLIGER, E *Die periphere Innervation* 1964 12th edn. Schwabe & Co Verlag 21 219 pp

WIBERG A *Function of the chorda tympani before and after operation for clinical otosclerosis* Dissertation Umea 1971 p 1—79

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Osmotic Fluid Transfer from Tissue to Blood during Hemorrhagic Hypotension

By

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Abstract

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Arterial and venous plasma osmolality and glucose concentration, and net transcapillary fluid movements were studied in the cat lower leg muscles *during hemorrhagic hypotension (50 mm Hg).* Arterial plasma osmolality increased rapidly after bleeding and exceeded after 20 min the control value by about 20 mOsm/kg H_2O, a level maintained during the remainder of the 90 min observation period. Popliteal venous osmolality rose much more slowly and did not reach the arterial level until some 50 min after the bleeding. Studies of plasma glucose concentration showed that the arterial and regional venous hyperosmolality almost entirely could be ascribed to a concomitant hyperglycemia, in particular during the first 30 min of hemorrhage. A clearcut arterio-venous osmolar difference in the muscle (reflecting a transcapillary osmolar gradient) was thus present in the early (≈50 min) stages of bleeding. In the *sympathectomized muscle region an absorption of extravascular fluid to the blood stream* occurred as long as this arterio-venous osmolar difference existed, strongly indicating that the fluid transfer was caused by osmosis. Comparative studies on the innervated and the sympathectomized muscle region showed that the extravascular fluid absorption due to reflex decrease of hydrostatic capillary pressure was most prominent in the early (< 20 min) stages of bleeding, whereas the osmotic fluid absorption was maintained for a more prolonged period of time. Both mechanisms no doubt are important for plasma volume restoration after hemorrhage.

In recent years hyperosmolality has been assigned important roles for the circulatory control in certain situations. Thus tissue hyperosmolality in skeletal muscle has been *shown to be one of the dominant mediators of exercise hyperemia* (Mellander *et al.* 1967, Lundvall 1972). Increased osmolality has furthermore been reported to evoke vasodilatation in several other vascular beds, such as the intestinal (Lundvall 1972), *coronary (Gazitua et al. 1971), cerebral (Wahl et al. 1973) and pulmonary* (Hauge and Bø 1971) circulation, although the functional significance of some of these effects remains to be clarified. Tissue hyperosmolality also has been shown to cause considerable plasma fluid transfer into the exercising muscles (Lundvall 1972), but the consequent decrease of plasma volume in this situation is much less than expected which, to a great extent, is due to a concomitant osmotic absorption of extravascular fluid from inactive tissues. The latter phenomenon is the result of an arterial hypero

molality caused by delivery of osmols from the exercising muscles (Lundvall *et al* 1972)

These results which revealed an osmotic control of plasma volume by agents other than the plasma proteins suggested that a similar compensatory mechanism might be operating in other situations as well and our preliminary observations (Järhult *et al* 1972) indicated that this indeed seemed to be the case in hemorrhagic hypotension Thus hemorrhage led to a pronounced arterial hyperosmolality in this case however mainly due to arterial hyperglycemia and to a transcapillary absorption of extravascular fluid from skeletal muscle

In the present study this problem was analysed in greater detail to permit a quantitative evaluation of the fluid redistribution between the extra and intra vascular compartments in hemorrhagic hypotension. The data suggest that the osmolar control of plasma volume is quantitatively as important as the previously well known reflex vascular and hormonal mechanisms (for ref see Chien 1967 Mellander and Johansson 1968) that tend to restore plasma volume during bleeding

Methods

Observations were made on 49 cats ranging in weight from 1.8 to 6.0 kg mean 3.2 kg) The animals were kept on a normal mixed diet before the experiment, except for the last 12 h when they were deprived of food They were anesthetized i.v. with α-chloralose 50 mg kg supplemented with pentobarbital sodium 10—30 mg 27 of the cats received urethane (100 mg kg as well. No difference in results could be observed between the two groups of animals given different anesthetics

General experimental procedure The study was performed on a region consisting of the [illegible] cles of the lower leg The skin was dissected free from the leg muscles the paw removed at the ankle joint and the thigh muscles separated from the lower leg The femur was drilled and plugged Small vessels between the thigh and lower leg were ligated so that the popliteal artery and vein formed the sole vascular connections with the main part of the body The sciatic nerve was left intact exception, see below The lower leg muscles covered with the dissected skin were placed in a waterfilled 34—35 C plethysmograph After heparin [illegible] 7.0 IE kg venous outflow from the region was diverted from the popliteal vein via an [illegible] drop-recorder [illegible] the external jugular vein The carotid artery was cannulated and connected to a siliconized graduated pressure bottle the pressure of which could be adjusted [illegible] so as to permit bleeding and lowering of arterial pressure to a constant level of 50 mm Hg Arterial pressure was monitored from a T tube in the cannula in the carotid artery Venous blood samples were taken from the popliteal vein and arterial samples from the carotid artery Arterial and venous plasma osmolality was measured by thermistor cryosc[illegible] Osmometer 31 LAS Advanced Instruments, Inc and plasma glucose by the plasma glucose [illegible] The animals breathed spontaneously throughout the course of the experiment

With this technique it was possible to follow the reactions within the different consecutive sections of the vascular bed for details see Mellander 1960 Mellander and [illegible] 19[illegible] The reactions of the resistance vessels were obtained from recordings of the [illegible] gradient and blood flow The response of the capacitance vessels which was [illegible] the response of the resistance vessels [illegible] Mellander 1960 was determined from the [illegible] initial decrease of tissue volume following bleeding and net transcapillary fluid movement [illegible] be obtained from the later and slower changes of tissue volume Variations [illegible] of the functional capillary surface area were estimated in some experiments from changes in the capillary filtration coefficient

When the surgical procedures were finished the animals rested for [illegible] 30 min before experimental intervention Venous outflow pressure was set so as to [illegible] an [illegible] state in the muscle region in order to achieve [illegible] normal [illegible] during which [illegible] transmural pressure After the period of rest another 10—15 min elapsed during which [illegible] arterial and venous blood samples were taken for determination of osmolality and glucose [illegible] the mean of these was taken as the resting control values The animal was then [illegible]

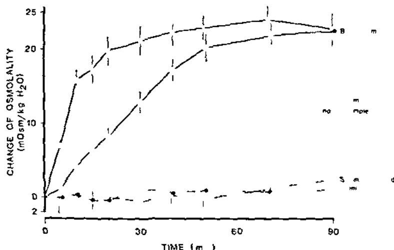


Fig 1 Changes from control value of arterial and popliteal venous plasma osmolality (mean values ± S E) in cats bled to 50 mm Hg (n = 45) and in sham operated cats (n = 4)

into the pressure bottle to an arterial pressure level of 50 mm Hg which was maintained throughout the period of hemorrhage Regional circulatory effects were followed during a hypotensive period of about 90 min during which repetitive blood samples were also taken for determination of osmolality and glucose These surgical and experimental procedures apply to the studies performed on the muscle region with intact sympathetic nerves (13 cats) and in principle also to the rest of the studies except for some special arrangements (see below)

After most experiments the lower leg muscles were dissected free from the bones for determination of muscle weight If not weighed the muscle re ion was considered to be 1 7 per cent of total body weight (Lundvall 197?)

In Reults section spread of data is expressed as S E

Sympathectomized muscle region In these experiments (14 cats) the sciatic nerve which contains virtually all sympathetic fibres to the lo er leg muscles was severed during the surgical preparation The arterial inflow to the muscle region was diverted from the proximal femoral artery via a T tube catheter to the popliteal artery Through this catheter dibenzyline (?—4 mg) was injected to block the α adrenergic receptors in the region To avoid systemic effects of the drug the regional venous outflow was collected for a few min afte the injection discarded and sub tituted by i v administration of the same volume of dextran The α receptor blockade was effective as judged by the absence of con trictor effects to noradrenal ne test injections The experimental procedures in these animals with surgical and pharmacological sympathectomy (below called sympathectomized reg on) were otherwise the same as described above

Sympathectomized pump pe fused m scle gi n In 9 cats the muscle region was sympathectomized as desc ibed above but the arterial inflow was controlled by means of a perfus on pump (Harvard model 1?10) nserted in the femo al popl t al hunt circuit. This permitted manual adjustments of egional blood flow to app ox mately the same lev ls as observed n the experiments w h nta t innervat on In oth espects the experimental protocol was the same as descr bed abo e

Animals not expos d to l a l g u g) In 9 cats arte al and femoral venous plasma osmolality was follow u for a 90 min p riod of hemo rhag c hypoten ion p oduced as described The hind paws wer excluded from the circulation by tight ligatures in these experiments but no lower leg s rg ry as performed

Sham operat d c t Four cats were subjected to the same kind of surg cal preparation as made on those with the sympathectomized muscle region These animal however were not bled after the u ual control period but left undistu bed n order to re eal pos ible spontaneou circulatory or o molar changes n the course of the ?0 min observation period

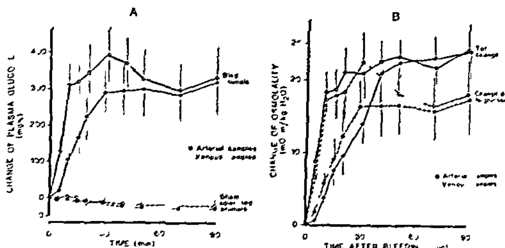


Fig 2 *A* Changes from control value of arterial and popliteal venous plasma glucose concentration in cats bled to 50 mm Hg (*n* = 11) and in sham operated cats (*n* = 4) *B* Changes above control value of total arterial and venous plasma osmolality in cats bled to 50 mm Hg (n = 11) shown together with deduced data for osmolality changes caused by concomitant alteration of plasma glucose concentration Mean values ± S E given

Results

Change of plasma osmolality Arterial and popliteal venous osmolality was observed in 45 cats during a 90 min period of hemorrhagic hypotension at 50 mm Hg. In the control period before bleeding mean arterial pressure was 120 ± 3 mm Hg, mean arterial plasma osmolality 318 ± 0.9 mOsm/kg H_2O (below abbreviated mOsm) and mean popliteal venous osmolality 319 ± 1.1 mOsm (in agreement with Lundvall 1972). The great majority of the animals showed clearcut increases of plasma osmolality during the period of hemorrhage and occasionally up to levels [illegible] mOsm above the control value. In 4 cats however no significant change of osmolality was observed. Fig 1 summarizes the results from all these experiments. It can be seen that after the beginning of hemorrhage (zero time) arterial osmolality increased rapidly to reach an average value of about 16 mOsm above control after 10 min and about 21 mOsm after 30 min, the latter level of hyperosmolality being maintained throughout the period of bleeding. Venous osmolality also rose but at a much slower rate and did not reach the arterial osmolality level until some [illegible] min after the commencement of the bleeding. The sham operated animals did not show any significant alteration of arterial or venous osmolality.

The pronounced arterio-venous osmolar difference observed during the first (≈ 40 min) period of hemorrhage shows that the plasma hyperosmolality must emanate at least mainly from some source other than the muscle tissue itself. This question is dealt with in the section below.

Change of plasma glucose concentration Data from the literature have indicated that hemorrhage is often associated with a rise of blood glucose concentration

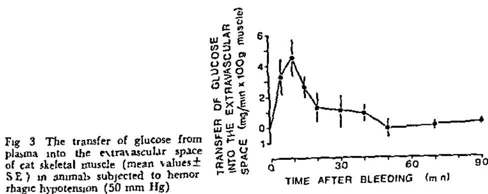


Fig 3 The transfer of glucose from plasma into the extravascular space of cat skeletal muscle (mean values ± S.E.) in animals subjected to hemorrhagic hypotension (50 mm Hg)

apparently caused by glucose release from the liver (for ref see Discussion) The possibility that the hyperglycemia contributes to the hyperosmolality during bleeding was examined by detailed analyses of plasma glucose and osmolality in arterial and popliteal venous blood during a 90 min period of hemorrhagic hypotension at 50 mm Hg (11 cats)

In the control period arterial plasma glucose averaged 216 ± 23 mg % and venous plasma glucose 206 ± 22 mg % Fig 2 A shows the changes of plasma glucose in the bled animals (n = 11) and in the sham operated animals (n = 4) The arterial plasma glucose increased rapidly after bleeding to reach a peak value of 394 ± 72 mg % above the control level after 30 min (maximal individual increase 806 mg %) after which there was a slight decline to a level of about 300 mg % above control maintained throughout the hypotensive period Popliteal venous plasma glucose increased much more gradually due to transfer of glucose into the extravascular space of the muscle and reached the arterial level in about 50 min Sham operated animals showed no significant change in arterial or venous plasma glucose concentration

Fig 2 B shows the extent to which the hyperglycemia may contribute to the concomitantly observed hyperosmolality For this deduction 18 mg % of glucose was considered to correspond to 1 mOsm/kg H_2O The diagram shows a close relation between the observed values for osmolality changes and those deduced from the glucose changes This close relation is most obvious in the early (≈ 30 min) period of bleeding when in fact the hyperglycemia seems to almost fully account for the plasma hyperosmolality

The curves for arterial and popliteal venous glucose concentration (Fig 2 A) indicate that an equilibration with the extravascular space of muscle occurred after about 50 min In Fig 3 the transfer of glucose into the extravascular space of muscle is calculated in approximate terms from the arterio-venous glucose difference times plasma flow (hematocrit considered to be 35 per cent due to fluid absorption (see below) instead of the normal value of 40 per cent (Altman and Dittmer 1971)) The net glucose transfer as expected was most rapid in the early phases of hemo-

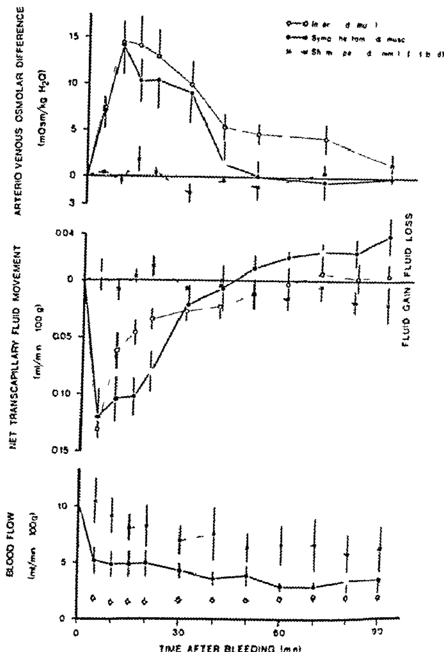


Fig 4 Arterio-venous osm l r difference net transcapillary flui l m o emen t at l reg r al l l d flow in an inn rvated (13) and a s mpathectn zed n 14 keletal m scle reg n 4 cats subjected to l em rrhag h poten ion (50 mm Hg) and n sham perated cat (n 4) Data expressed as mean lues ± S E

rhage and ceased after about 50 min despite the fact that the arterial glucose level was still high and blood flow in this phase was maintained at an approximately constant level (see below)

Transcapillary fluid movements Since in early hemorrhage the tissue is perfused by blood which is hyperosmolar (hyperglycemic) a transcapillary osmolar gradient would be established that might cause a net transcapillary fluid absorption from the extravascular to the intravascular space The latter question was approached by studying the net transcapillary fluid flux the arterio-venous osmolar difference and the regional blood flow in the sympathectomized muscle (14 cats) as well as in the innervated muscle (13 cats) during hemorrhagic hypotension at 50 mm Hg The observed *arterio-venous osmolar difference* may be *considered to reflect in rough* terms the prevailing transcapillary osmolar gradient (*cf* Lundvall 1972)

Fig 4 shows the collected data from these experiments as well as from the sham operated animals It can be seen (upper panel) that the arterio-venous osmolar difference established during hemorrhage was relatively similar in the innervated and the sympathectomized muscle region A peak value for this arterio-venous difference of about 14 mOsm was reached after 10 min and it then subsided gradually No significant alteration was present in the sham operated animals

The middle panel of Fig 4 shows the concomitant net transcapillary fluid movement (ml/min × 100 g tissue) derived from the observed change of tissue volume after correction for the capacitance response occurring in the initial period of bleeding (see Methods section) This capacitance response was 0 34 ± 0 05 ml/100 g in the innervated and 0 09 ± 0 02 ml/100 g tissue in the sympathectomized skeletal muscle The rate of net transcapillary fluid absorption reached a peak value after about 5 min both in the innervated (0 131 ± 0 008 ml/min × 100 g) and the sympathectomized muscle (0 120 ± 0 030 ml/min × 100 g) and then gradually declined In the latest stages of hemorrhage there was a tendency to a reversal with some fluid loss into the extravascular space The sham operated animals showed only minor net transcapillary fluid movements Note that the time courses of the curve for arterio venous osmolar difference and for net transcapillary fluid absorption are quite similar especially in the sympathectomized muscle region indicating that the two phenomena are causally linked to each other These results and some other observations (see Discussion) seem to make it clear that the arterial hyperosmolality in hemorrhage causes considerable osmotic fluid absorption into the blood stream

The finding that the fluid absorption was less pronounced in the innervated than in the sympathectomized muscle during the period 10 to 30 min after the commencement of bleeding seems *a priori* somewhat surprising in view of the fact that besides the described osmotic fluid flux a vascular reflex fluid absorption is known to occur in the intact muscle during bleeding (*e g* Lundgren *et al* 1964) Osmotic transcapillary fluid absorption however has recently been shown to depend both on the arterio venous osmolar difference (transcapillary osmotic gradient) *and* blood flow (Lundvall 1972) and regional blood flow in the present study was as

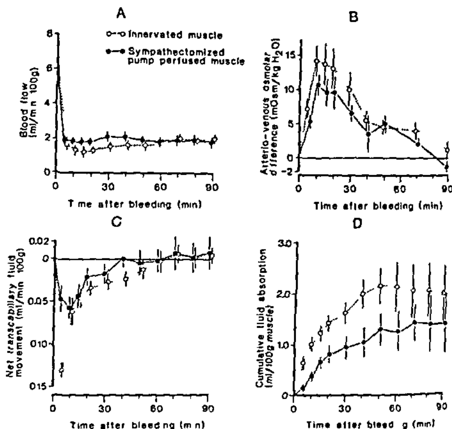


Fig 5 Comparative data in hemorrhage for arterio-venous osmolar difference (panel B) and transcapillary fluid movement (panel C) and cumulative fluid absorption from the extravascular space (panel D) in the innervated (n = 13) and sympathectomized (n = 9) muscle region when blood flow in the latter region was mechanically adjusted to about the same level as occurred in the innervated one (panel A). Data for innervated muscle (panel A—C) same as in Fig 4. Mean values ± S.E. given

considerably higher in the sympathectomized than in the innervated muscle (Fig 4 lower panel). Control blood flow was 50 per cent larger in the sympathectomized than in the intact muscle and during the hypotensive period flow was 2—4 times higher in the former than the latter. Mention should also be made that the sham operated animals showed some gradual decline of flow during the observation period an effect apparently due to some derangement secondary to the surgical trauma

Since as mentioned osmotic fluid absorption appears to be flow dependent some experiments (n = 9) were performed on the sympathectomized pump perfused muscle region in which the arterial inflow of blood could be adjusted to about the same level as was observed during hemorrhage in the innervated muscle (Fig 5 A). Fig 5 B shows that the magnitude and the time course of the evoked changes in arterio-venous osmolar difference were quite similar in the pump perfused and the innervated muscle experiments. Under these circumstances when blood flow and the arterio-venous osmolar difference were almost identical the observed fluid absorp-

tion in the pump perfused muscle region may be considered to represent roughly the osmotic fraction of the total transcapillary fluid absorption present in the intact muscle during hemorrhage see Fig 5 C (As will be discussed below the somewhat lower (10—20 mm Hg) arterial inflow pressure in the pump perfused than in the autoperfused muscle does not lead to a lowering of capillary hydrostatic pressure under the prevailing experimental conditions) The data in Fig 5 C thus shows the approximate extent to which osmosis (closed circles) contributes to the total absorption process (open circles) The cumulative fluid absorption in the intact muscle (reflecting total absorption) and in the pump perfused muscle (reflecting osmotic absorption) *is shown in Fig 5 D After 10 min the total amount of absorbed extra* vascular fluid in 100 g of muscle averaged 1 02 ml ($\approx$ 35 per cent osmotic) after 20 min 1 43 ml ($\approx$ 55 per cent osmotic) and after 60 min 2 13 ml ($\approx$ 60 per cent osmotic)

For a deduction of the effective transcapillary driving forces which cause the fluid transfer during bleeding (see Discussion) the capillary hydrodynamic conductivity (filtration coefficient) must be known The filtration coefficient determined in some of the experiments was found to vary between 0 018—0 023 ml/min $\times$ 100 g tissue $\times$ mm Hg in different stages of hemorrhage

Discussion

In this investigation the shock model of Wiggers (1942) was used which implies bleeding to a constant level of arterial hypotension in this case 50 mm Hg With this approach *the arterio-venous pressure gradient in the studied region is maintained* constant throughout the period of hemorrhage which is advantageous for detailed studies of net transcapillary fluid movements The analysis of such phenomena was furthermore facilitated in the present experiments by blockade of the lymph drainage from the studied region This shock model however creates an artificial hemorrhage situation in so far that the animal cannot benefit from the effects of certain compensatory mechanisms that normally would tend to restore blood pressure Thus an animal with vivid compensatory adrenergic constrictor reflexes will be exposed to a larger hemorrhage and a more intense peripheral vasoconstriction than one with less efficient sympatho adrenal system (*cf* Chien 1967) This was noticed in the present experiments in terms of a relatively wide range of the volume of shed blood the fraction of total blood volume that had to be withdrawn to achieve a blood pressure of 50 mm Hg varied in the different animals from 11—31 per cent (mean value 24 ± 1 6) Even if therefore a relatively large spread of data might be expected the present main results were quite consistent Hemorrhage in most cases led to a pronounced arterial hyperosmolality (Fig 1) mainly due to hyperglycemia (Fig 2) which caused a transcapillary osmotic fluid absorption from the extra vascular space of skeletal muscle into the circulatory system (Fig 4 and 5)

There are some previous reports in the literature on the existence of a clearcut *plasma hyperosmolality in hemorrhage (e g* Bergentz and Brief 1965 Baue *et al*

1967 Boyd and Mansberger 1968, Boyd *et al* 1970) as well as occasional reports of small or negligible increases (Brooks *et al* 1963, Schwinghamer *et al* 1970 Gregg *et al* 1971) Hyperglycemia after hemorrhage was first described by Claude Bernard (1877) and has later been repeatedly confirmed in different species such as the dog (*e g* Sato *et al* 1928 Brooks *et al* 1963 Baue *et al* 1967, Bauer *et al* 1969) the cat (*e g* Brooks 1935) the sheep (Halmagyi *et al* 1966), the rat (*e g* Engel *et al* 1943) the baboon (*e g* Moss *et al* 1970) and man (*e g* Boyd and Mansberger 1968 Carey *et al* 1970) The present study however, seems to be the first one aimed at an investigation of the possible role of hyperosmolality and hyperglycemia in plasma volume regulation during bleeding

In the present series of experiments hemorrhage led to a pronounced hyperosmolality in about 90 per cent of the animals maintained throughout the observation period of 90 min The hyperosmolality could be ascribed mainly to hyperglycemia (Fig 2 B) at least during the first 30 min of bleeding

The main cause of hyperglycemia in hemorrhage evidently is an increased output of glucose from the liver (*e g* Selye 1950 Shoemaker *et al* 1961) and this effect in turn has been mainly attributed to the increased adrenaline release (*e g* Sato *et al* 1928 Watts 1956 Walker *et al* 1959) Adrenalectomy or administration of ganglionic blocking agents has been reported to prevent the hyperglycemia after hemorrhage (*e g* Engel *et al* 1943 Halmagyi *et al* 1966 1967) Yet the direct adrenergic innervation may also contribute to the response since in adrenalectomized animals stimulation of the splanchnic nerves can lead to release of glucose from the …er (Edwards 1971) Other hormones such as glucagon (Halmagyi *et al* 1969) and …h hormone (Cerchio *et al* 1971) have not been ascribed any major role in the hemorrhagic hyperglycemia The insulin response evidently is species specific In some including man (Carey *et al* 1970) the insulin level seems virtually unaffected whereas in others such as the dog (Bauer *et al* 1969 McCormick *et al* 1969) it rises but yet not so as to abolish the hyperglycemia

The release of glucose to the blood stream is an effect which starts early after the commencement of the bleeding and an arterial plasma glucose peak value is reached after some 30 min (Fig 2 A) A dilution of the released glucose of course occurs primarily in the intravascular and secondarily in the interstitial compartment The arterial and venous glucose concentration curves (Fig 2 A) indicate that an approximate steady state concentration in the extracellular space is obtained in about 1 h The tendency to declining arterial glucose concentration in the later (> 30 min) stages of bleeding might at least partly be ascribed to a gradually decreasing glucose release The hyperglycemic response to hemorrhage was quite pronounced also in those animals in the present series of experiments that were subjected to a relatively small bleeding suggesting that this response is evoked already by moderate stimulation of the adrenal medullary secretion

The data in Fig 2 B show that the plasma hyperosmolality in early (< 30 min) hemorrhage almost entirely could be ascribed to the hyperglycemia This does not rule out the possibility that other constituents of the blood may change but then

apparently in different directions so as to largely cancel an effect on osmolality In later stages however the maintained hyperosmolality in face of a somewhat declining hyperglycemia indicates that in this period other factors also contribute to the osmolar change

Studies of transcapillary fluid movements in the sympathectomized and *a* receptor blocked muscle region showed that net fluid absorption from the extravascular space occurred as long as an arterio venous osmolar difference (transcapillary osmolar gradient) was present The very similar time course of these two events (Fig 4 solid lines) strongly suggests that the transcapillary absorption was due to osmosis Seemingly the only alternative explanation for the fluid absorption would be a fall of hydrostatic capillary pressure secondary to the reduced arterial inflow pressure However other studies in skeletal muscle have clearly shown that as long as vascular reactivity is maintained a decrease of arterial pressure *per se* does not lead to a fall of capillary pressure when as in this case venous outflow pressure is kept constant (Lewis and Mellander 1962 Öberg 1964) Thus autoregulation of capillary pressure in muscle is present at least during the first hour of reduced arterial inflow pressure and is apparently due to precise adjustments of the pre /postcapillary resistance ratio in skeletal muscle (Lewis and Mellander 1962 Since in the present experiments with pump perfusion the arterial inflow pressure was quite low special control experiments in normovolemic cats were performed in which arterial inflow pressure to the muscle region was *mechanically* reduced to the same low levels ≈ 30 mm Hg (Jarhult unpublished) In these experiments tissue volume remained virtually constant during an observation period of 90 min indicating that capillary hydrostatic pressure was not lowered if anything there was a slight *filtration* during the last hour Postcapillary sludging may contribute to this somewhat unexpected phenomenon —Since in the present hemorrhage experiments on sympathectomized muscle the fluid absorption was mainly occurring within the first hour of hemorrhagic hypotension (Fig 4) the conclusion thus is that the fluid flux was related to osmosis

The effective transcapillary osmotic driving force was however relatively small as calculated from the observed rate of fluid absorption divided by the value for the capillary filtration coefficient Five min after the start of the bleeding the driving force was about 6 mm Hg after 10 min about 3 mm Hg and then it gradually declined Yet as discussed below the hemodynamic consequences of the osmotic process can be quite important

Although the observed osmotic fluid absorption is a transcapillary event it is quite likely that part of the absorbed fluid primarily emanated from the intracellular space due to osmotic dehydration of the skeletal muscle fibres It cannot be entirely ruled out then that the transcapillary hydrostatic gradient is somewhat changed if osmotic withdrawal of intracellular fluid lead to increased interstitial pressure

Previous studies by Lundvall (1972) have indicated that transcapillary osmotic fluid movement is a phenomenon which is blood flow dependent. This view seemed corroborated by the present results showing a decreased fluid flux in the sympathectomized muscle region when blood flow was mechanically reduced to the same

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caused by delivery of osmols from the exercising muscles (Lundvall *et al* 1972) Such an osmotic control of plasma volume is of special importance in heavy exercise with large muscle groups to compensate for a very marked plasma fluid loss into the active muscles The net plasma volume decrease in this situation is limited to a great extent by an osmotic fluid gain from inactive tissues

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References

ALTMAN P L and D S DITTMAR (ed) Respiration and circulation *Biological Handbooks* Federations of American Societies for Experimental Biology Bethesda Maryland 1971 p 2?5

BAECKSTROM P B FOLKOW A G B KOVACH B LOFVING and B ÖBERG Evidence of plugging of the microcirculation following acute haemorrhage *6th Europ Conf Microcirc Aalbo g* 1970 Ed by J Ditzel and D H Lewis Karger Basel 1971 pp 16—2?

BAUE A E E T TRAGUS and W M PARKINS Effects of increased osmolality and correction of acidosis on blood flow and oxygen consumption in hemorrhagic shock *J Surg Res* 1967 *7* 349—356

BAUER W E S N M VIGAS R E HAIST and W R DRUCKER Insulin response during hypovolemic shock *Surgery* 1969 *66* 80—88

BERGENTZ S E and D K BRIEF The effect of pH and osmolality on the production of canine hemorrhagic shock *Surgery* 1965 *58* 412—419

BERNARD C *Leçons sur le Diabete et la Glycogenese Animale* Paris Baillere 1877 p 210

BOYD D R and A R MANSBERGER JR Serum water and osmolal changes in hemorrhagic shock An experimental and clinical study *Amer Surg* 1968 *34* 744—749

BOYD D R F A FOLK R E CONDON L M NYHUS and R J BAKER Predictive value of serum osmolality in shock following major trauma *Surg Forum* 1970 *21* 32—33

BROOKS C M The reaction of chronic spinal animals to hemorrhage *Amer J Physiol* 1935 *114* 30—39

BROOKS D K W G WILLIAMS R W MANLEY and P WHITEMAN Osmolar and electrolyte changes in haemorrhagic shock *Lancet* 1963 *1* 521—5?7

CAREY L C B D LOWEREY and C T CLOUTIER Blood sugar and insulin response of humans in shock *Ann Surg* 1970 *17?* 34?—350

CERCHIO G M G S MOSS P A POPOVICH E BUTLER and D C SIEGEL Serum insulin and growth hormone response to hemorrhagic shock *Endocrinology* 1971 *88* 138—143

CHIEN S Role of the sympathetic nervous system in hemorrhage *Physiol Rev* 1967 *47* 214—288

CHIEN S Blood rheology and its relation to flow resistance and transcapillary exchange with special reference to shock *Advanc Microcirc* vol ? Karger Basel/New York 1969 pp 89—103

EDWARDS A V The glycogenolytic response to stimulation of the splanchnic nerves in adrenalectomized calves sheep dogs cats and pigs *J Physiol* (Lond) 1971 *213* 741—749

ENGEL F L M G WINTON and C N H LONG Biochemical studies on shock I The metabolism of amino acids and carbohydrate during hemorrhagic shock in the rat *J Exp Med* 1943 *77* 397—410

ERIKSSON E and B LISANDER Low flo tates n the m o e ls of skeletal muscle in cat *Acta physiol scand* 1972 *86* ?0?—?10

GAZITUA S J B SCOTT B SWINDALL and F J HADDY Resistance r pon es to local change in plasma osmolal t in th a c l b d l m *J Physiol* 1971 *220* 384—391

GRECA G I I M SCHW t IAMER and F J HADDY Changes in forelimb weight and segmental vascular resistances following se e e hemorrhag *C ulat R s* 1971 *29* 691—703

HALMAGYI D F J D J GILLET L LAZARUS and J D YOUNG Blood gluco e and se u n insulin in reversibl and irre e ible posthemorrhag c shock *J Trauma* 1966 *6* ?23—629

HALMAGYI D F J M H IRVING and D J GILLETT Cou e of posthemorrhagic hypoten ion after celiac ganglionectomy with and without adrenal denervation *J S rg* 1967 2??—??7

level as in the innervated region (compare Fig 4 middle panel and Fig 5 C) The latter figure also shows the net transcapillary fluid absorption during hemorrhage in the innervated muscle region, in this case the fluid transfer must be ascribed partly to osmosis and partly to the well known reflex decrease of hydrostatic capillary pressure (*e g* Öberg 1964 Lundgren *et al* 1964 *cf* also Mellander and Johansson 1968) The relative importance of these two mechanisms for transcapillary fluid absorption in skeletal muscle can be seen from Fig 5 C and also from the curves for cumulative fluid transfer presented in Fig 5 D The total fluid absorption as observed in the innervated muscle (Fig 5 D) is of about the same order of magnitude as previously described during moderate hemorrhage (Lundgren *et al* 1964) The absorption caused by reflex decrease of capillary pressure was dominant in the early stages of hemorrhage but with time the osmotic process became relatively more pronounced In 60 min the total fluid absorption amounted to 2 1 ml/100 g muscle tissue of which some 1 3 ml could be attributed to osmosis

Hemorrhage in the human being is reported to lead to a plasma hyperosmolality of similar magnitude to the one described here for cats (Boyd and Mansberger 1968 Boyd *et al* 1970) Extrapolation of the present data on transcapillary fluid absorption to man suggests that in moderate hemorrhage some 650 ml would be absorbed from all skeletal muscles (≈ 30 kg) in the first hour after bleeding of which some 400 ml may be attributed to osmosis Studies in man of the net increase of plasma volume resulting from intravenous infusion of hypertonic glucose solution have shown that the gained fluid is retained within the circulatory system for considerable length of time after the cessation of the hypertonic infusion (Lundvall *et al* 1972)

The present results considered in view of the discussion above indicate the osmolar control of plasma volume to be an important compensatory mechanism in hemorrhage

The fact that the fluid absorption from muscle declined with time and finally ceased (Fig 4) may be explained by several factors *a* gradual decrease of the reflexly increased pre-/postcapillary resistance ratio due to declining precapillary but maintained postcapillary adrenergic constriction (Lewis and Mellander 1962 Mellander and Lewis 1963 Lundgren *et al* 1964 Eriksson and Lisander 1972) *b* increased postcapillary resistance due to rheological changes on the venous side (increased viscosity cell aggregation etc see Chien 1969 Baeckstrom *et al* 1971 Eriksson and Lisander 1972) *c* gradual decrease of the transcapillary osmotic gradient (Fig 4) and *d* gradual decline of the plasma protein concentration due to absorption of protein poor extravascular fluid It is possible that under circumstances when venous outflow pressure is not maintained constant but permitted to fall as a consequence of vascular derangement and decreased filling of the circulatory system transcapillary fluid absorption can be prolonged simply due to a passive fall of capillary hydrostatic pressure (*cf* Schwinghamer *et al* 1970 Grega *et al* 1971)

An osmolar control of plasma volume due to agents other than plasma proteins has previously been described also in another situation *i e* in exercise when an arterial hyperosmolality also develops In this case however the arterial hyperosmolality is

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The Influence of Blood Flow on the Rate of Absorption of ^{85}Kr from the Small Intestine of the Cat

By

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Abstract

BIBER B O LUNDGREN and J SVANVIK *The influence of blood flow on the rate of absorption of ^{85}Kr from the small intestine of the cat* Acta physiol scand 1973 *89* 227—238

A method for studying the absorption rate of an easily diffusable substance ^{85}Kr from the small intestinal lumen to the intestinal blood stream is described and tested The technique involves a fast, luminal perfusion with saline having a constant concentration of ^{85}Kr and a continuous registration of the radioactivity in the venous outflow from the intestine Absorption rate is estimated from the amount of tracer appearing in the mesenteric vein and expressed as volume of blood that per unit time is fully equilibrated with the luminal contents During resting conditions (total intestinal blood flow 25 ml/min × 100 g) the absorption rate was about 5 ml/min × 100 g and during a moderate hyperemia (total intestinal blood flow 100 ml/min × 100 g) induced by isopropylnoradrenaline absorption rate increased to about 13 ml/min × 100 g The results are discussed in relation to the small intestinal mucosal blood circulation and countercurrent exchanger

The relationship between total intestinal blood flow and rate of intestinal absorption has been studied by several authors Thus the absorption of such basic nutrients a glucose (Williams Mager and Jacobson 1964 Varro *et al* 1965) and amino acids (glycine Varro *et al* 1965) was found to be impaired when the intestinal blood flow was reduced However since the O_2 consumption and ATP content of the intestinal mucosa was concomitantly reduced the impaired absorption was ascribed to a reduced oxygen supply to the actively absorbing epithelial cells (Varro *et al* 1965 Varro 1966 Winne 1971 a) The absorption of water has also been found to be dependent on the intestinal blood flow (Nelson and Beargie 1965 Winne 1972) as well as the absorption of a number of pharmacological substances (for ref see Winne 1971 a)

The elimination of gaseous CO_2 from the intestinal lumen was investigated by Pals and Steggerda (1966) finding a retarded disappearance at a reduced intestinal blood flow while Hamilton Dawson and Webb (1968) could not correlate the disappearance rate of intraluminal ^{133}Xe dissolved in an isotonic fluid with the blood

flow. In a study by Coburn (1968) the elimination of CO from the intestinal lumen was on the other hand found to be highly dependent of blood flow.

In all the abovementioned studies changes in total intestinal blood flow were induced without knowing how the experimental procedures affected the blood flow in the superficial layers of the mucosa where absorption takes place. Since mucosal hemodynamics in the small intestine of the cat was recently analysed in detail during various experimental conditions (Biber *et al.* 1973, Biber, Lundgren and Svanvik 1973, Lundgren and Svanvik 1973, Svanvik 1973), it was considered of interest to correlate directly the absorption of an easily diffusable substance to the blood flow in the intestinal mucosa proper. The complex vascular bed of this tissue includes a villous countercurrent exchanger proposed to delay the absorption of particularly easily diffusing solutes (Lundgren 1967, Kampp, Lundgren and Sjöstrand 1968) and also a plasma skimming mechanism creating a low hematocrit in the villous blood vessels (Jodal and Lundgren 1970). Since one primary interest of the present study was to get information about the influence of the mentioned countercurrent exchanger on intestinal absorption, ^{85}Kr, earlier shown to be shortcircuited in the countercurrent exchanger when applied via the blood stream, was chosen as a test substance. Furthermore ^{85}Kr offers the advantage of being both metabolically inert and largely excluded from the circulation after a single pulmonary passage.

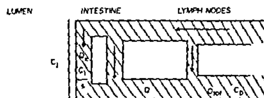
The absorption of ^{85}Kr was studied both during "rest" and during intestinal hyperemia induced by isopropylnoradrenaline and the experimental conditions were tested by different control experiments. In a subsequent paper the effects on ^{85}Kr absorption induced by reductions in perfusion pressure and by vasoconstrictor fibre stimulations will be reported.

A preliminary report of parts of this study was published elsewhere (Lundgren and Svanvik 1968).

Methods

A. Theoretical considerations. Winne (1961, 1971 b) has outlined the theory for the movement of substances from the lumen of the small intestine to the intestinal blood stream, using theoretical models with two to four compartments. In the present study a simplified experimental model was chosen by using an inert gas (^{85}Kr) as the tracer under circumstances when the intraluminal concentration of the tracer is largely constant, the amount of tracer transported *via* the venous effluent being equal to the amount leaving the lumen. In such model experiments it is possible to discuss absorption in a system made up by two main compartments, lumen and blood (see Fig. 1), however interposed by an unknown number of not defined compartments.

The absorption of the tracer was measured from its appearance in the vein draining the intestinal preparation. The ^{85}Kr concentration in the lumen (C_l) was kept largely constant by a fast, luminal perfusion of saline equilibrated with ^{85}Kr. The venous effluent (Q_{v}) from the intestinal segment including its mesentery and lymph nodes was continuously measured as was the tracer concentration (C_v) in this blood. From these measurements it is possible to calculate the tracer amount $C_v \times Q_v$, per time unit appearing in the venous blood and to relate this to C_l. The expression $C_v \times Q_v$ then denotes the intestinal rate of absorption, since ^{85}Kr is almost completely eliminated from the blood during its passage through the lungs and the arterial concentration of ^{85}Kr is hence also negligible. The rate of ^{85}Kr absorption can be regarded to be proportional to C_l, all other factors being kept constant. Thus $C_v \times Q_v/C_l$ constitutes a measure of the "absorption capacity" of the intestine. In analogy with the clearance concept in the kidney the expression $C_v \times Q_v/C_l$ also denotes the volume of the intraluminal fluid that per unit time is "cleared" of the tracer.



Q venous outflow from the intestin
Q amount of blood p time unit fully quilibrated w h he luminal contents
Q venous outflow from the int tine lymph nodes
C_l luminal ^{85}Kr oncentration
C_b ^{85}Kr concentration in the venou ffluent f om intestine lymph nodes
water/blood partition oefficient for ^{85}Kr

$$Q = \frac{Q_{tot} \; C_b}{C_l}$$

Fig 1 Hypothetical model of the ^{85}Kr absorption from the small intestinal lumen to the intestinal blood stream For details see text

Since the main interest of the present study was to relate the rate of absorption to intestinal blood flow the most useful way to express the absorptive capacity was found to be in terms of the volume of blood that per unit ti ne was fully equilibrated with the luminal contents The following discussion is based on the hypothetical model hown in Fig 1 The concentration of an eas ly diffus ble tracer in the blood which is fully equilibrated with the luminal concentra tion C_l can be calculated to be C_l/s s being the water blood partition coefficient If the hypothetical blood flow that is equilibrated with the luminal concentration C_l is denoted Q the amount of tracer absorbed can be expressed as $Q_x \times C_l/s$ w h ch in turn equals the amount absorbed *via* the blood

Thus

$$Q \quad \times \frac{C_l}{s} = Q_t \quad \times C_b \tag{1}$$

$$Q \quad = \frac{Q_{t\,t} \times C_b \times s}{C_l} \tag{2}$$

By comparing Q to the intramural blood flow of the intestine (Q) it is possible to calculate the fraction (Q /Q) of intramural blood flow that is fully equilibrated with the luminal contents

B Ope ati e procedures and determination of bl d flow The experiments were performed on 17 cats anesthetized with chlo alose (40—60 mg/kg b w) after ether induction The animals had been deprived of food at least 24 h and had no obvious signs of intestinal infect on

The operative p ocedu es have earlier been described in detail (f Folkow Lundgren and Wallentin 1963 Kampp Lundgren and Sjost and 1968 see also Fig 2 The abdomen was opened in the midline and the greater omentum and the pleen v ere extirp t d Th spleen was first electrically stimulated to expell its blood A segment of the jejunum weigh ng 10—30 g was isolated and the remainder of the intestinal tract extirpated The lumen of the jej nal segment was flushed with bodywarm Tyrode olut on or al ne until a clear fluid emerged The mesentery and its vessels was covered by M lar (Du Pont to h nder d ffusion of the tracer from tissue to air The influence of the s mp thico-adrenal ystem as eliminated by bilateral ection of the splan hnic nerves by den rv tion of the left adrenal gland and b ex l sion of the right one fro n blood circulation b ligat ire The agal nfluence was eli n nated by atropine 1—1 5 mg/kg b w

After heparinizat on th ight f n oral artery a conne ted to a m r ury manon ete o to a Statham pres ure t an du e to re o d mean arterial pressure (Fig 2) The up rior mesent ric vein drain ng all bl od from th jejunal segm nt and its l mph nodes was nn l ted t a glass tube leading th blood nto a ell t e sc nt llat on det cto and ubseq ently into an optical drop-r ord —ordinat writer unit onti uously regi t r ng enou o flow Q B occlud ng the es els of th n entery t was po bl to rrea r th blood flo n th l mph nodes and part of the m sentery eparately and thus to deduce the int am ral blood flow (Q) of the intest ne pr per V no is o itflow p e sure was measu ed and u ally et to 10 mm Hg but could be set at desired levels b djusting th end of th tube drai ng the drop reco de

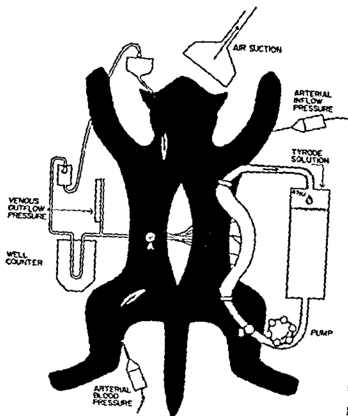


Fig 2 Schematic illustration of the technique used for studying rate of absorption of ^{85}Kr from the small intestinal lumen of the cat For details, see text

The venous blood was returned to the animal via a funnel and a catheter in the right jugular vein A small branch of the superior mesenteric artery was cannulated for i a infusions of isopropyl noradrenaline to induce graded intestinal vasodilatations

C Isotope technique and luminal perfusion system Both ends of the intestinal segment were cannulated with glass tubes connected to a mantled glass reservoir containing about 200 ml saline and about 40 ml gas at a constant temperature of 38 C A plastic tube and a pump (Sigmamotor or Harvard pulsatile pump) were interposed between the reservoir and the glass cannula supplying the intestine with perfusate as regulated by the pump and varied between 0—140 ml/min (see Fig 2 About 10 mCi of Kr dissolved in 11 ml of saline was added to the perfusion system. The intraluminal volume of the intestinal loop was kept largely constant by adding or eliminating saline from the system Care was taken to avoid intestinal distension by a too large volume of fluid in the otherwise rigid perfusion system

Thus the intestinal perfusion system constituted a closed circuit with a constant volume and an almost constant ^{85}Kr concentration in the fluid since it was continuously equilibrated with the gas phase having a tracer concentration 20 times that of the perfusate Samples of 1 ml of the perfusion fluid and venous blood could be intermittently taken in glass syringes at the points labelled A and B in Fig 2 Its content of γ radioactivity was measured in a well type scintillation detector coupled to a spectrometer (Packard Auto-Gamma Spectrometer series 410 A) and a scaler The radioactivity of the venous blood was continuously measured in a second well type scintillation detector operating another spectrometer coupled to a rate meter and an inkwriter

D Control experiments In order to quantitate the possible diffusion of the tracer from the tissue into the surrounding air the following experiments were performed In three cats prepared as described in section B above the lumen of the intestinal segment was flushed with saline and emptied Care was taken to avoid air bubbles in the lumen The superior mesenteric artery was then cut and its distal part cannulated with a glass cannula connected to a glass syringe with 120 ml of a dextran Tyrode solution containing about 1 mCi of ^{85}Kr In this way

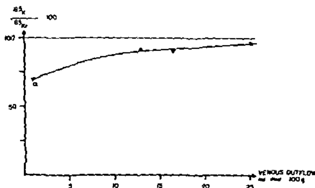


Fig 3 Data from three control experiments where the small intestinal vascular bed was perfused at various rates with a dextran Tyrode solution having a constant ^{85}Kr concentration, $^{85}Kr_a$ The tracer concentration in the venous effluent $^{85}Kr_v$ was continuously measured The venous concentration expressed in per cent of the arterial is plotted *vs* venous outflow Different registrations refer to different experiments Lines drawn by inspection

the vascular bed could be perfused at various rates with a fluid having a constant concentration of ^{85}Kr The radioactivity in the venous outflow was continuously measured and could be compared to the radioactivity level of the perfusion fluid of the syringe which was determined by infusing the fluid directly into the cannula draining the mesenteric vein

E Autoradiographic technique In an attempt to study the distribution volume in the tissue of the absorbed tracer an autoradiographic study was performed using antipyrine N methyl ^{14}C (*New England Nuclear Chemicals*) ^{85}Kr was not suitable for this part of the study because of its comparatively high energy β radiation and the difficulties inherent in avoiding its evaporation from thin tissue slices The autoradiographic technique was similar to that used by Kety on the brain (personal communication see also Kampp *et al* 1968) Into the closed perfusion system (see section C) 100 μCi of antipyrine N methyl ^{14}C was injected After about 10 min of intraluminal perfusion at 35 or 50 ml/min the lumen of the intestinal segment was first rapidly flushed with 5 ml saline and then immediately extirpated and frozen in liquid nitrogen The frozen segment was sliced in a cryostate (System Dittes Duspiva) maintained between −20 C and −25 C into 10 or 30 μm thick transverse slices The sections were mounted on glass and heated to 65—70 C for about 30 s They were then placed in close contact with dental X ray film (Ilford dental x ray film standard no 5) and exposed at room temperature together with a drying agent (silica gel) for varying periods of time the time dependent on the amount of radioactivity in the tissue The localisation of the tracer in the intestinal wall as indicated by the blackness of the autoradiographs was determined by simultaneous microscopical examination of the histological section and its corresponding autoradiograph

Control experiments to rule out any marked diffusion artefact in this autoradiographic technique has been reported earlier (Kampp *et al* 1968)

F Calculations The rate of absorption of ^{85}Kr was calculated from equation 2 and expressed in ml/min × 100 g The water blood partition coefficient (volume/volume) s is dependent on the hematocrit (hct) of blood and this was corrected for using the equation

$$s = \frac{188}{0.63 \times hct - 137} \qquad (3)$$

which is based on the experimental data reported by Lassen and Munkh (1955) It has been demonstrated that the hematocrit in the mucosal vessels of the cat intestine is only 60 per cent of that in arterial blood (Jodal and Lundgren 1970) a fact that was considered when using equation 3

Results

A Methodological control experiments In order to test to what extent the absorbed ^{85}Kr evaporated from the tissue before reaching the portal vein intestinal segments were perfused with a dextran Tyrode solution containing ^{85}Kr as described in Methods section D The experiments were performed at low venous outflows (below 25 ml/min × 100 g) since it was believed that such low flow rates would

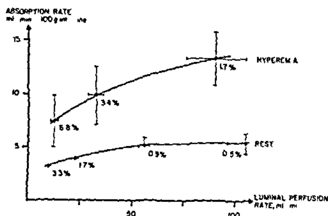


Fig 4 Cumulated data on the relationship between luminal perfusion rate and ^{85}Kr absorption rate during "resting" blood flow (6 expts mean venous outflow of the different points 29—31 ml/min × 100 g) and during hyperemia induced by isopropyl noradrenaline (5 expts mean venous outflow of the different points 84—91 ml/min × 100 g) The absorbed fraction of the total amount of tracer passing the lumen is indicated at each point. Lines drawn by inspection Bars indicate ± S E

fractional tracer losses by means of tissue to air diffusion When the ^{85}Kr concentration in the venous effluent had reached a constant value after 10—20 min perfusion it was compared with that in the arterial inflow The results are illustrated in Fig 3 showing that the tracer concentration in the effluent was more than 90 per cent of that of the inflow fluid when the intestinal flow exceeded 13 ml/min × 100 g tissue At flows above 20 ml/min × 100 g which represent a flow level usually encuntered in the resting cat intestine the loss of tracer was negligible If the Mylar® covering both sides of the mesentery was taken away the tracer loss increased about 50 per cent at low flow rates

In four experiments it was checked whether the ^{85}Kr concentration really was negligible in arterial blood It was in these controls found that the arterial radio activity level was less than one per cent of that in the intestinal venous outflow

The extent to which the rate of luminal perfusion affected the ^{85}Kr resorption was studied in 8 experiments Fig 4 shows the accumulated data on the absorption rate as plotted vs the luminal perfusion rate both at resting blood flow levels (6 expts) and during hyperemia where intestinal blood flow was increased 3—4 times to about 85 ml/min × 100 g by ia infusion of isopropylnoradrenaline (5 expts) It

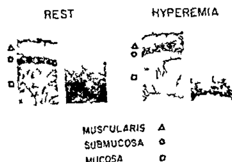


Fig 5 Cat 2 8 kg Autoradiographs (right) and corresponding histological sections (left) of two intestinal segments which had been perfused intraluminally for 10 min with a saline solution containing antipyrine N methyl ^{14}C Experiments were performed during resting conditions and during intestinal vasodilatation induced by isopropylnoradrenaline Exposure time for autoradiographs Resting blood flow segment 18 days hyperemia segment 22 days

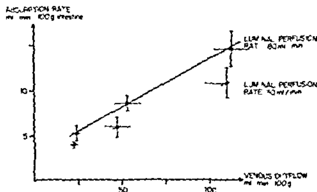


Fig 6 Cumulated data on the correlation between total intestinal blood flow as varied by i a infusions of isopropyl noradrenaline and the rate of ⁸⁵Kr absorption during low (7 expts) and high luminal perfusion rate (7 expts) Lines drawn by inspection Bars indicate ± S E

can be seen that the rate of absorption increased with the luminal perfusion rate During resting intestinal blood flow the absorption rate levelled off when the luminal perfusion rate exceeded 50 ml/min During hyperemia the absorption rate was more dependent upon the luminal perfusion but showed a tendency to level off at very high intraluminal flows

The figures given in Fig 4 indicate the tracer amount absorbed in per cent of the total tracer amount passing through the intestinal lumen It is evident that at high rates of luminal perfusion the extraction of radioactivity due to absorption amounted to only 1—2 per cent

In order to study the distribution of an easily diffusible tracer within the intestinal wall an autoradiographic study was performed using the inert solute antipyrine N methyl ^{14}C as described in Methods section E Autoradiographs were obtained both at resting blood flow levels and during intense vasodilatation Representative autoradiographs and corresponding unstained histological sections are shown in Fig 5 The exposure time in the two experiments of Fig 5 was so chosen as to give the same blackening of the muscularis It is apparent that the radioactive material is confined

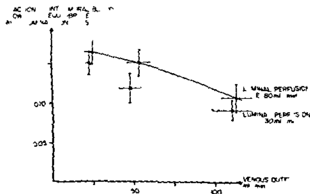


Fig 7 The relationship between total intestinal blood flow and fraction of intramural blood flow fully equilibrated with the luminal contents The calculation were performed on data from the same experiments as shown in Fig 6 Lines drawn by inspection Bars indicate ± S E

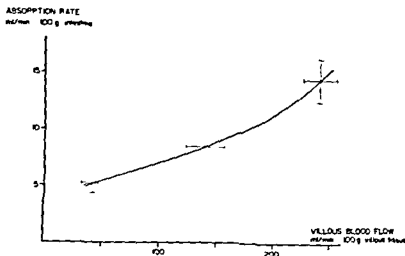


Fig 8 ⁸⁵Kr absorption rate plotted *versus* villous blood flow as varied by i.a. infusions of isopropylnoradrenaline The Figure is based on the experimental data of the upper curve of Fig 6 Villous blood flow was estimated from the correlation between intestinal venous outflow and villous plasma flow obtained in a previous study (Biber Lundgren and Svanvik 1973) For details see text Line drawn by inspection. Bars indicate ± S E

to the villi at vasodilatation while largely the entire mucosa is blackened at "resting" flow levels Thus the distribution volume of antipyrine N methyl ¹⁴C is decreased intestinal blood flow is increased

B Rate of absorption of ⁸⁵Kr versus blood flow The steady state correlation between total intestinal blood flow and rate of ⁸⁵Kr absorption is illustrated in Fig 6 the measurements being performed at two different levels of intraluminal perfusion rate Each curve is based on 7 expts and in 4 of these the ⁸⁵Kr absorption was studied at both rates of luminal perfusion The results show that the rate of ⁸⁵Kr absorption is largely proportional to the intestinal blood flow within the range studied These results have in Fig 7 been used for calculations of the fraction of intestinal blood flow that becomes fully equilibrated with the intestinal contents (cf Methods section A) It can be seen that this fraction becomes smaller the larger the intestinal blood flow

In a previous publication (Biber Lundgren and Svanvik 1973) the villous plasma flow was determined at various levels of total intestinal blood flow as changed by i.a infusions of isopropylnoradrenaline Using these results as corrected for the comparatively low hematocrit in the villous vessels (Jodal and Lundgren 1970) it was possible to correlate the rate of ⁸⁵Kr absorption to villous blood flow Such calculations were performed on the experiments shown in Fig 6 and 7 and the results are shown in Fig 8 As villous blood flow is augmented a proportionally larger increase of absorption rate is seen particularly when villous blood flow exceed 150 ml/min × 100 g

Discussion

The present investigation was carried out to test the reliability of an experimental model aimed at studying the relationship between intestinal blood flow and rate of absorption from the intestinal lumen. Moreover the effect on absorption rate of varying intestinal blood flow by means of a vasodilator drug was investigated.

The model was evaluated by testing experimentally the theoretical assumptions. Thus as was pointed out in the theoretical part of Methods (section A) it was assumed that (1) the tracer concentration in the arterial blood was negligible and that (2) no concentration gradients existed in the intestinal lumen as long as it was properly perfused. Further during steady state conditions it was supposed that (3) the amount of tracer leaving the intestinal lumen was identical to that appearing in the venous effluent from the intestine.

Ad 1 The arterial concentration of the absorbed ^{85}Kr would *a priori* be expected to be very low since first the intestinal venous blood becomes greatly diluted when reaching the large veins and the heart and second because ^{85}Kr is to about 85 % eliminated in the lungs (Chidsey *et al* 1959). The control experiments confirmed this view since the arterial ^{85}Kr concentration was less than one per cent of that in the venous effluent from the intestine and thus could be regarded as negligible.

Ad 2 The possibility of concentration gradients within the intestinal lumen was tested by studying the effect of varying the luminal perfusion rate on absorption (Fig 4). It was found that the absorption rate increased when the luminal perfusion was augmented. However a level of luminal perfusion was eventually reached at least at low blood flows where a further increment did not effectively increase the absorption rate. These results may be explained by several mechanisms, one being that the high rates of luminal perfusion cause an effective perfusion also of the intervillous spaces thus exposing a larger intestinal surface area to the perfusate. With the present techniques it is not possible to differentiate between such a factor and the hypothetical existence of intraluminal concentration gradients at low perfusion rates that were eliminated when the intraluminal flow increased. Finally it may be argued that mechanical stimulations of the intestinal mucosa activate a local vasodilator mechanism (Biber, Lundgren and Svanvik 1971) which might possibly be triggered by a high rate of luminal perfusion. However no increases of intestinal blood flow were seen upon increments of luminal perfusion which speaks against this latter possibility.

Regardless of the mechanism responsible for the results of Fig 4 one may conclude that a high rate of luminal perfusion is necessary to eliminate intraluminal factors and thus to make possible a study only of the factors that are involved in transport of ^{85}Kr from the lumen to the mucosal blood stream.

Ad 3 The radioactivity level of the intestinal venous outflow was continuously monitored and was assumed to represent the total amount of absorbed tracer. Since ^{85}Kr is not metabolized such an assumption may seem correct but at least two sources of error must be considered. First the tracer amount situated extravascularly in the intestinal tissue, *i e* the distribution volume, may be altered by differen-

perimental procedures as demonstrated in Fig 5 If for example the distribution volume would increase the tracer amount in the vein would be less than that leaving the intestinal lumen To avoid such errors measurements were performed during steady state conditions, as reflected by unchanged recorded parameters

Second the possibility exists that during the transit from the intestinal mucosa to the portal vein part of the tracer leaves the blood stream It was also demonstrated (Fig 3) that a slight tracer escape could occur when the vascular tree of an intestinal segment was perfused with a fluid containing ^{85}Kr particularly at low flows However when the intestine is autoperfused by blood and the tracer enters at the mucosal capillary level the ^{85}Kr loss would be even less than that shown in Fig 3

To summarize the theoretical assumptions for the use of the present technique seems to be largely fulfilled although a slight tracer loss by evaporation may occur at very low blood flows and further intraluminal concentration gradients may exist at high blood flows

According to the autoradiographic study illustrated in Fig 5 it is evident that during resting conditions all tracer diffusing from the lumen into the tissue is not transported away by the villous subepithelial capillary network since almost the entire mucosa was blockened by the labelled tracer During the same conditions ^{85}Kr was absorbed at a rate of 5 ml/min $\times$ 100 g intestine meaning that only 17 per cent of total intestinal blood flow was fully equilibrated with the luminal contents This fraction does not even exceed the fraction of intramural blood flow distributed to the villi which during rest is usually about 20 per cent (Biber Lundgren and Svanvik 1973) and is considerably lower than the fraction of intramural blood flow pass the mucosa (45 per cent) There are reasons to presume that during absorption of ^{85}Kr the villous blood flow should be fully equilibrated with the luminal tracer concentration (see below) and that the blood flow in the deeper parts of the mucosa should be at least partly equilibrated to this concentration Thus a far higher fraction than the observed 17 per cent of the intramural blood flow should be expected to be fully equilibrated with the luminal contents

During infusion of isopropylnoradrenaline increasing total intestinal blood flow from 25 to 100 ml/min $\times$ 100 g the ^{85}Kr absorption rate was increased while the fraction of intestinal blood flow fully equilibrated with the luminal contents decreased steeply to about 0 12 (Fig 6 and 7) The autoradiographic findings during intestinal hyperemia indicated that the tracer now was confined to the villi probably because at this high blood flow the mucosal tissue constituted a relatively more important diffusion barrier that now hindered the diffusion into the deeper parts of the mucosa It was earlier demonstrated (Biber Lundgren and Svanvik 1973) that 30 to 35 per cent of the intramural blood flow passes the villous capillaries at a total intestinal blood flow of 100 ml/min $\times$ 100 g Thus only 35—40 per cent of the villous blood flow will be fully equilibrated with the luminal content

From the discussion above it can be concluded that there is a considerable discrepancy between the absorption rate and the intestinal mucosal blood flow both during resting blood flow and during hyperemia It might be argued that the

could be due to a diffusion limitation for ^{85}Kr across the epithelial cells (height 25 μm). However, it can be estimated using Fick's law that ^{85}Kr (assumed diffusion coefficient 2×10 cm /s) in 0.25 s will reach a 97 per cent equilibrium across a 25 μm thick sheet of water (see Davson 1970, page 397). The mean transit time for plasma in the villous hairpin vascular loops, mainly spent in the subepithelial capillary network, exceeds 1.5 s even during a hyperemia (Biber, Lundgren and Svanvik 1973), a time long enough for a full equilibration of ^{85}Kr between lumen and villous blood.

Other mechanisms must be sought for and it is proposed that the discrepancy between villous blood flow and rate of absorption of ^{85}Kr is to a large extent caused by the countercurrent exchange mechanism proposed to exist in the mucosa by Lundgren (1967). According to this hypothesis the net blood absorption of krypton will be delayed due to its diffusion from the subepithelial capillaries and/or venules to the mucosal arterial vessels. Thus the inert gas will be "recirculated" towards the villous tips. A similar "hindrance" for net blood transport was made likely by Lundgren and coworkers (Lundgren 1967) when introducing krypton into the intravascular compartment. Such a countercurrent exchange diffusion from artery to vein and/or capillary seemed, however, to be considerably reduced when intestinal blood flow was increased above 150 ml/min × 100 g by vasodilatation. The linear rate of flow in the mucosal vascular loops then became so fast that the time available for exchange diffusion became critically reduced. On the other hand, when an easily diffusible substance is aproaching the mucosal exchanger from the "lumenal" side the exchanger is probably much more eficiently "hindering" net blood transport of a substance due to the far slower linear rate of flow in the capillaries as compared to that in the central arterial vessels of the villi (*cf.* Niesel and Roskenbleck 1963). It seems difficult to estimate quantitatively the importance of the countercurrent exchanger at the various intestinal flow rates since many other factors of importance for absorption, *e.g.* the "distribution" volume of the tracer in the tissue, are affected by changing blood flow. The results of Fig. 8 may, however, possibly indicate that the "efficiency" of the countercurrent exchanger decreases with increasing blood flow even when approached from the luminal side since absorption rate increased out of proportion to villous blood flow in the higher range of flows.

The results described and discussed above are in agreement with earlier reports in the literature concerning the absorption of easily diffusible solutes. Thus Grim, Lee and Visscher (1955) reported that only 5 per cent of total intestinal blood flow became fully equilibrated with D_2O placed in the lumen. Furthermore, Hamilton, Dawson and Webb (1967) showed that the corresponding figure for ^{133}Xe was 2—3 per cent. In the latter experiments the intestinal segment was not perfused with the ^{133}Xe solution and the low absorption rate may to some extent be explained by the existence of intraluminal concentration gradients.

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References

BIBER B O LUNDGREN and J SVANVIK Studies on the intestinal vasodilatation observed after mechanical stimulation of the mucosa of the gut *Acta physiol scand* 1971 *82* 177—190

BIBER B O LUNDGREN and J SVANVIK Intramural blood flow and blood volume in the small intestine of the cat as analysed by an indicator-dilution technique *Acta physiol scand* 1973 *87* 391—403

BIBER B O LUNDGREN L STAGE and J SVANVIK An indicator-dilution method for studying intestinal haemodynamics in the cat *Acta physiol scand* 1973 *87* 433—447

CHIDSEY C A H W FRITTS A HARDEWIG D W RICHARDS and A COURNAND Fate of radioactive krypton (Kr) introduced intravenously in man *J appl Physiol* 1959 *14* 63—66

COBURN R F Carbon monoxid uptake in the gut *Ann NY Acad Sci* 1968 *150* 13—21

DAVSON H *A textbook of general physiology* Ed IV 1970 Vol 1 J and A Churchill London

FOLKOW B O LUNDGREN and I WALLENTIN Studies on the relationship between flow resistance capillary filtration coefficient and regional blood volume in the intestine of the cat *Acta physiol scand* 1963 *57* 270—283

GRIM E J S LEE and M B VISSCHER Water exchange between intestinal contents tissues and blood *Amer J Physiol* 1955 *182* 359—363

HAMILTON J D A M DAWSON and J WEBB Limitation of the use of inert gases in the measurement of small gut mucosal blood flow *Gut* 1967 *8* 509—571

JODAL M and O LUNDGREN Plasma skimming in the intestinal tract *Acta physiol scand* 1970 *80* 50—60

KAMPP M O LUNDGREN and J SJOSTRAND The distribution of intravascularly administered lipid soluble substances in the mucosa and the submucosa of the small intestine of the cat *Acta physiol scand* 1968 *72* 469—480

LASSEN N A and O MUNCK The cerebral blood flow in man determined by the use of radioactive krypton *Acta physiol scand* 1955 *33* 30—49

[L]UNDGREN O Studies on blood flow distribution and countercurrent exchange in the small [intest]ine *Acta physiol scand* 1967 Suppl 303

[LU]NDGREN O and J SVANVIK Uptake of [85]Kr from the lumen of the small intestine to the intestinal blood in the cat *Acta physiol scand* 1968 *74* 20 A—21 A

[L]UNDGREN O and J SVANVIK Mucosal hemodynamics in the small intestine of the cat during reduced perfusion pressure *Acta physiol scand* 1973 *88* 551—563

[N]ELSON R A and R J BEARGIE Relationship between sodium and glucose transport in canine jejunum *Amer J Physiol* 1965 *208* 375—379

[R]IESEL W and H ROTTENBLECK Die Bedeutung der Stromgeschwindigkeiten in den Gefässsystemen der Niere und der Schwimmblase fur die Aufrechterhaltung von Konzentrationsgradienten *Pflugers Arch ges Physiol* 1963 *277* 302—315

[SE]ALS D T and F R STEGGERDA Relation of intra intestinal carbon dioxide to intestinal blood flow *Amer J Physiol* 1966 *210* 893—896

[S]VANVIK J Mucosal haemodynamics in the small intestine of the cat during regional sympathetic vasoconstrictor activation *Acta physiol scand* 1973 *89* 19—29

VARRO V *Beziehungen zwischen Resorption und Blutzirkulation am Duenndarm Internist* (Berl) 1966 *5* 250—255

VARRO V G BLAHO L CSERNAY I JUNG and F SZARVAS *Effect of decreased local circulation on the absorptive capacity of a small intestine loop in the dog Amer J dig Dis* 1965 *10* 170—177

VARRO V I JUNG F SZARVAS L CSERNAY G SAVAY and J OKROS The effect of vasoactive substances on the circulation and glucose absorption of an isolated jejunal loop in the dog *Amer J dig Dis* 1967 *12* 46—59

WILLIAMS J H M MAGER and E D JACOBSON Relationship of mesenteric blood flow to intestinal absorption of carbohydrates *J Lab clin Med* 1964 *63* 853—863

WINNE D and H OCHSENFAHRT Die formale Kinetik der Resorption unter Berucksichtigung der Darmdurchblutung *J theor Biol* 1967 *14* 293—315

WINNE D Durchblutung und enterale Resorption *Z Gastroenterologie* 1971 a *6* 429—441

WINNE D Die Pharmakokinetik der Resorption bei Perfusion einer Darmschlinge mit variabler durchblutung *Naunyn Schmiedebergs Arch exp Path Pharmak* 1971 b *268* 417—433

WINNE D The influence of blood flow and water net flux on the absorption of tritiated water from jejunum of the rat *Naunyn-Schmiedebergs Arch exp Path Pharmak* 1972 *272* 417—436

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The Effect of Reduced Perfusion Pressure and Regional Sympathetic Vasoconstrictor Activation on the Rate of Absorption of ^{85}Kr from the Small Intestine of the Cat

By

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Abstract

SVANVIK J *The effect of reduced perfusion pressure and regional sympathetic vasoconstrictor activation on the rate of absorption of ^{85}Kr from the small intestine of the cat* Acta physiol scand 1973 *89* 239—248

The absorption from the small intestinal lumen to the intestinal blood stream of an easil diffusable substance ^{85}Kr was studied in the cat during different hemodynamic conditions. When reducing arterial inflow pressure to the intestine from about 100 to 30 mm Hg the absorption rate was reduced in proport on to the venous outflow although an earlier study demonstrated an almost unchanged villous blood flow during corresponding experimental conditions Also when raising the venous outflow pressure absorption rate decreased in propor tion to venous outflow while during neurogenic reductions of intestinal blood flow the absorption rate was not significantly changed The results are discussed mainly with regard to the intestinal mucosal hemodynamics and countercurrent exchanger

Mechanical interference with the arterial inflow to the small intestine is known to influence absorption Thus Varro *et al* (1965) found a reduced active absorption rate for glucose and glycerol from the small intestine of the dog during graded obstruction of the supplying artery Since a concomitant reduction of the O consumption was recorded the decreased absorption rate was ascribed to an impaired function of the absorbing cells The passive resorption of sorbose was on the other hand not correlated to the reduct on of the blood flow Similarly Nelson and Beattie (1965) noted an impaired absorption of water and glucose upon aortic compression in the dog and Winne (1972) reported a reduced intestinal absorption of a variety of test substances in the rat upon reducing the intestinal blood flow by bleeding

It has also been shown that an increased portal pressure can influence the absorption Thus Shields and Code (1961) found an impaired sodium absorption at increased portal pressures in dogs and Lee and Duncan (1967) in the rat noted a

reduced water transport from lumen to blood when the intestinal venous outflow pressure was raised

The adrenergic influence on the intestinal absorption has also been studied by several authors Winne (1966) and Varro *et al* (1967) report reduced absorption rates of tritiated water and glucose respectively, during intravascular administration of catecholamines On the other hand, stimulation of the sympathetic vasoconstrictor fibres increased the disappearance rate of glucose from the small intestinal lumen in the cat (Huidobro Valdes and Davila 1947)

Thus there are many studies indicating that changes of intestinal haemodynamics influence intestinal absorption though little is known about the relationship between mucosal blood flow and intestinal absorption since methods have been lacking for quantitating the blood supply to the mucosa A recently developed indicator dilution technique (Biber *et al* 1973) has however made possible a fairly detailed study of mucosal haemodynamics in the small intestine of the cat also during reduced perfusion pressure (Lundgren and Svanvik 1973) and stimulation of the regional vasoconstrictor fibres (Svanvik 1973) Furthermore a method for studying the absorption of krypton from the intestinal lumen has been presented (Biber Lundgren and Svanvik 1973) and it was regarded of interest to use this method to study absorption during experimental conditions where known changes of mucosal blood flow were induced Hence below are reported experiments during stimulation of the intestinal sympathetic nerves and during reductions of the perfusion pressure, induced either by lowering arterial or raising venous pressure Preliminary results from part of these studies have earlier been reported (Lundgren and Svanvik 1968)

Methods

The experiments were performed on 17 cats anesthetized i v with chloralose (50—70 mg/kg b w) and deprived of food for at least 24 h. The experimental approach as closely similar to that described in an earlier paper (Biber Lundgren and Svanvik 1973) which also gives details regarding calculation of the absorption expressed as ml of blood which per unit time becomes fully equilibrated with the luminal contents

To summarize the method involves a continuous registration of venous outflow (Q_{tot}) from an isolated acutely denervated intestinal segment *in situ* by means of an optical drop recorder unit. The lumen of the segment was perfused at a high rate with body warm saline with a nearly constant ^{85}Kr concentration thus largely avoiding concentration gradients in the intestinal lumen The mesentery and its vessels were covered by Mylar® (Du Pont) to hinder diffusion of the tracer from tissue to air The radioactivity of the venous blood (C_b) was continuously measured by a well type scintillation counter and by taking concomitant samples of the luminal perfusate and the venous blood The absorption rate could be calculated as $C_b \times Q_{tot}$ and compared to the luminal tracer concentration (C_l) A value (Q_x) denoting the amount of blood in ml/min × 100 g that was fully equilibrated to the intestinal contents could also be calculated as $Q_x = C_t \times Q_{tot} \times s / C_l$ where s represents the water/blood partition coefficient for ^{85}Kr

Arterial inflow pressure registered from a small branch of the intact superior mesenteric artery was varied in steps of 20—30 mm Hg by means of a clamp around the mesenteric artery Venous outflow pressure was continuously measured and could be set at any desired level by adjustments of the end of the tube draining the drop recorder Absorption was determined at three different levels of venous pressure i e 6 5 13 and 33 cm H_2O

The nerves surrounding the superior mesenteric artery were dissected free and cut and the peripheral ends were mounted on a bipolar silver electrode Nerve stimulation was performed with square wave pulses (10 V 5 ms and 1—16 Hz) using a Grass stimulator Atropine 1—1.5

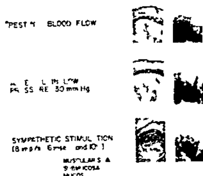


Fig 1 Cat 2 8 kg Autoradiographs (right) and corresponding histological sections (left) of three intestinal segments which had been perfused intraluminally for 10 min with a saline solution containing antipyrine N methyl ^{14}C Experiments were performed during resting conditions during reduced arterial inflow pressure and during the steady state phase at sympathetic stimulation Exposure time for the autoradiographs 18 days

ml/kg b w was given i v to block the concomitantly activated vagal cholinergic fibres to the intestine

By transient occlusion of the mesenteric vessels blood flow through the mesenteric lymph nodes could be measured separately and was subtracted from the venous outflow thus making it possible to determine the intestinal intramural blood flow Knowing this the fraction of the intramural blood flow that was fully equilibrated with the luminal contents could be calculated

To study the distribution volume in the tissue of an easily diffusible absorbed tracer an autoradiographic study was performed Since ^{85}Kr was not suitable for this another lipid soluble inert tracer antipyrin N methyl ^{14}C (New England Nuclear Chemicals) was used instead in the luminal perfusate The localisation of the tracer in the intestinal wall as indicated by the blackness of the autoradiographs as determined by simultaneous microscopical examination of the histological sections and the corresponding autoradiographs

Results

A The intramural distribution of antipyrin N methyl ^{14}C

In order to study the distribution of an easily diffusible tracer within the intestinal tissue during absorption an autoradiographic study was performed as described by Biber Lundgren and Svanvik (1973) Autoradiographs were obtained at resting blood flow at low perfusion pressure induced by a reduction of the arterial inflow pressure and during the steady state phase of sympathetic vasoconstrictor stimulation Fig 1 shows that during all of these experimental conditions of the intestinal vascular bed the distribution volume of antipyrin N methyl ^{14}C during absorption includes almost the entire mucosa

B Rate of ^{85}Kr absorption versus intestinal blood flow

1 The effect of lowering arterial inflow pressure Fig 2 illustrates a typical experiment in which the effect of a reduced arterial pressure on intestinal blood flow and venous radioactivity was studied The lumen of the gut was continuously perfused by a saline solution containing a constant concentration of ^{85}Kr and arterial pressure was kept lowered from a control value of 110 at about 25 mm Hg for 17 min It can be seen that the radioactivity of the venous outflow was if anything slightly reduced during the period of hypotension When however the arterial clamp was released the transient hyperemia was paralleled by a sharp rise in

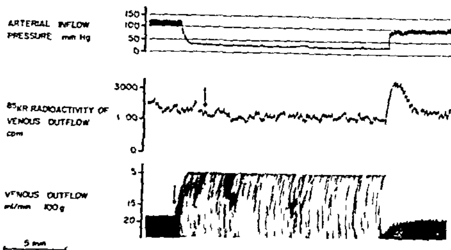


Fig 2 Cat 2 6 kg A representative experiment demonstrating the effect of a reduction of arterial inflow pressure to an intestinal segment on the venous outflow and on the venous ^{85}Kr radioactivity level The intestinal lumen was perfused with a saline solution containing a constant concentration of ^{85}Kr The registration of radioactivity was delayed as compared to venous outflow since intestinal blood did not immediately reach the scintillation detector Arrows indicate concomitant events in the two lower registrations The flow scale is approximate at the flow level during reduced pressure Venous outflow then varied between 4 5 and 5.5 ml/min × 100 g

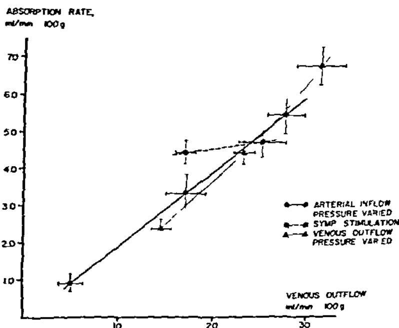


Fig 3 Cumulated data on ^{85}Kr absorption rate expressed as volume of blood that per unit time fully equilibrates with the luminal contents vs venous outflow from the intestinal segment Three different experimental situations were studied Arterial inflow pressure variations (6 expts.) stimulation of the regional sympathetic nerves 9 expts) and venous outflow pressure variations (6 expts.) Lines drawn by inspection. Bars indicate ± S E

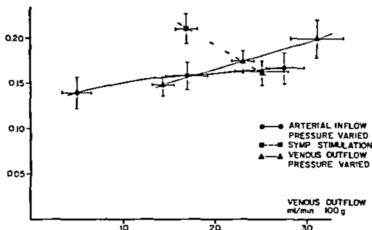


Fig 4 Cumulated data on the fraction of intestinal intramural blood flow that was fully equilibrated with the luminal contents *vs* venous outflow from the intestinal segment The curves are based on the experiments of Fig 3 Lines drawn by inspection Bars indicate ± S E

venous tracer concentration exceeding the prehypotensive control level Similar responses were seen al o after more moderate reductions of arterial pressure

In six experiments performed a shown in Fig 2 arterial pressure was reduced in steps of 20—30 mm Hg from a control level around 100 mm Hg to 20—30 mm Hg each step lasting 15—25 min Venous outflow pressure was kept constant at 10 mm

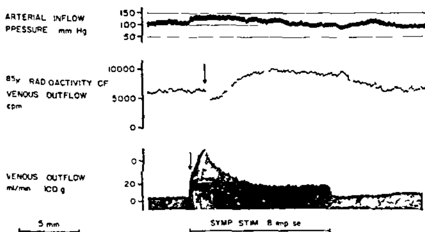


Fig 5 C t 2 1 kg A typical experiment d mon trating the effe t of a st mulation of the regional sympath et c nerves (8 Hz 10 V 5 ms) on total nte t nal blood flow and on nous Kr rad oact it level Th intestinal segment was perfused with a l n sol tion containing a con tant con ntrat n of Kr Arrows indicate oncom tant even in th two lo er registra tions at the beg nning of th n rve t mulat on

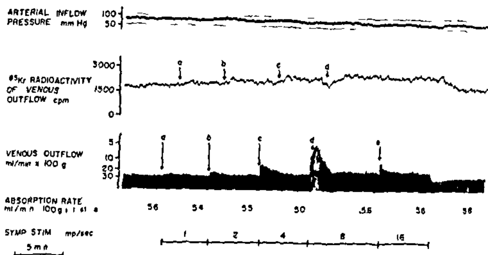


Fig 6 Cat 2 4 kg The effect of stimulation of the regional sympathetic vasoconstrictor fibres (7 V 11 ms) at various frequencies on arterial blood pressure total intestinal blood flow and venous ^{85}Kr radioactivity The experimental conditions were similar to those of Fig 2 and 5 Arrows indicate concomitant events in the two lower registrations Absorption rate during the steady phase of vasoconstriction is indicated in the lower part of the figure

Hg The absorption rate expressed as the amount of blood (ml/min × 100 g intestine) fully equilibrated with the intestinal contents was calculated and plotted against intestinal blood flow (Fig 3) The luminal perfusion rate was in most of these experiments above 45 ml/min a rate known to eliminate intraluminal concentration gradients during resting blood flow (Biber Lundgren and Svanvik 1973) From Fig 3 it is evident that the absorption rate decreases almost linearly with venous outflow

In Fig 4 the same experiments as in Fig 3 were used for calculations of the fraction of intestinal blood flow fully equilibrated with the luminal contents (cf Methods) From Fig 4 it can be seen that there is no significant change of this fraction if anything it is slightly reduced at very low flows

II The effect of raising venous outflow pressure In 6 expts venous outflow pressure was changed from a control value of 13 to 6 5 or 33 cm H O in a randomized manner In Fig 3 the absorption rate during these procedures is plotted against intestinal blood flow It can be seen that the absorption rate is almost linearly related to venous outflow as venous outflow pressure is varied The fraction of the intramural blood flow fully equilibrated with the luminal contents was calculated in the same experiments and is plotted vs intestinal blood flow in Fig 4 A slight decrease of the fractional absorption rate is observed when venous outflow pressure is raised When venous pressure was suddenly reduced from 33 to 6 5 cm H O the radioactivity in the venous outflow did not exceed the control although a considerable transient venous outflow increase was observed (cf Fig 2)

III The effect of regional vasoconstrictor fibres stimulation In nine atropinized cats the nerves surrounding the superior mesenteric artery were electrically stimulated In Fig 5 a representative experiments is shown where the effects of vasoconstrictor fibre stimulation (8 Hz 10 V 5 ms) on arterial pressure on total intestinal blood flow and on the radioactivity level of the venous outflow were studied The intestinal lumen was perfused at high rate with a fluid containing a more or less constant concentration of ^{85}Kr The characteristic blood flow response is seen in the lower part of the figure with the initial strong but transient vasoconstriction followed by an autoregulatory escape from the vasoconstrictor fibre influence and a stabilization at a flow level moderately below control Upon cessation of stimulation a slight transient hyperemia is observed The radioactivity level of the venous outflow is seen in the middle registration After an initial transient decrease the radioactivity level increases above control and stabilizes at a constant level during the steady state phase of vasoconstriction When stimulation is ended, no increase of radioactivity was observed despite some post stimulatory hyperemia Even after 20 min stimulation periods venous radioactivity was still above control

In four experiments nerve stimulations were performed at different frequencies (1 2 4 8 and 16 Hz) during consecutive five min periods (Fig 6) The level of total intestinal blood flow during the steady state phase is maximally reduced at 8 Hz and is not further reduced by a stimulation frequency of 16 Hz Concomitantly venous radioactivity is gradually increased showing a detectable increase even at a stimulation frequency of 1 Hz reaching its maximum at 8 Hz The calculated absorption rate shown in the lower part of the figure did not significantly change during these procedure

The calculated average absorption rates during rest and during the steady state phase of sympathetic vasoconstriction at 8 Hz ($n = 9$) are illustrated in Fig 3 It is evident that neurogenic blood flow reductions lead to only slight reductions of absorption rate, implying that the fraction of intramural blood flow fully equilibrated with the luminal contents is significantly increased (Fig 4)

Discussion

In recent studies the mucosal haemodynamics of the cat small intestine was explored during reduced perfusion pressure (Lundgren and Svanvik 1973) and during regional sympathetic stimulation (Svanvik 1973) In the present investigation the absorption of ^{85}Kr during the same conditions was studied in order to elucidate the complex relation between mucosal blood flow and intestinal absorption

During *reductions of arterial inflow pressure* an almost linear relationship was obtained between the intestinal absorption rate and intestinal blood flow (Fig 3) while the fraction of intramural blood flow fully equilibrated with the luminal contents stayed rather constant around 0 15 despite a comparatively large range of intestinal blood flow (Fig 4) A small decrease of this fraction was however noted at very low blood flows possibly caused by tracer evaporation as earlier discussed (Biber

Lundgren and Svanvik 1973) These results should be compared with recent haemodynamic findings (Lundgren and Svanvik 1973) where villous plasma flow remained largely constant even when arterial pressure was lowered from 100 to 30—40 mm Hg revealing a remarkable autoregulation of the villous vessels Simultaneously the fraction of total intestinal plasma flow distributed to the villi increased from 0 20 to 0 35—0 40 This means that the fraction of villous blood that fully equilibrates with the luminal contents becomes more reduced the lower the pressure head

The apparently contradictory observations described above cannot be reconciled using any of the current models of blood flow and/or diffusion limitation used when discussing e g the elimination kinetics from gas in subcutaneous gas pockets (see e g Piiper Canfield and Rahn 1962) The only mechanism that seems to offer a plausible explanation for the present observations seems to be the countercurrent exchange proposed to exist in the intestinal mucosa by Lundgren and coworkers (see e g Lundgren 1967) According to this hypothesis the present results are explained as follows The absorbed krypton is during resting conditions trapped in the countercurrent exchanger and therefore to a certain extent hindered from being transported away via the blood Although villous plasma (blood) flow stays largely constant because of autoregulation and hence an increased fraction of the intestinal blood supply is distributed to the villi the countercurrent exchanger becomes at the same time more efficient in hindering net blood absorption of the tracer The reason is that the linear rate of flow in the hairpin vascular loops is decreased facilitating the extent of crossdiffusion between the descending and ascending limbs Thus mean transit time in this part of the intestinal vasculature was reduced from 4—6 s at rest to 20—30 s during arterial hypotension at 30 mm Hg Hence absorption rate of krypton becomes decreased in the face of a largely unaltered villous blood flow It should in this connection be underlined that countercurrent exchange diffusion of easily diffusable solutes may occur also between the mucosal vessels of the crypts as reported by Kampp Lundgren and Sjostrand (1968) and possibly also in the submucosa thus explaining the distribution of antipyrin N methyl ^{14}C in the autoradiographs (Fig 1)

The transient rise of the radioactivity in the venous outflow seen after a period of hypotension may be explained by a reactive hyperemia in the villous vessel as demonstrated by Lundgren and Svanvik (1973) The sudden rise of pressure leads to a transient rise in linear flow rate in these vascular loops thus reducing the impeding effect on the absorption excerted by the countercurrent exchanger

During changes of the venous outflow pressure the absorption rate showed a similar relationship to blood flow as that observed upon reduction of arterial pressure (Fig 3) Also the fraction of intestinal blood flow fully equilibrated with luminal contents was similar to that observed during reduced arterial pressure (Fig 4) The haemodynamic effects of raising venous pressure on the mucosal vessels include a moderately decreased linear flow rate in the hairpin vascular loops due to the reduced perfusion pressure The fraction of the intramural plasma flow passing villi is however only slightly increased (Lundgren and Svanvik 1973) and the reduced

absorption rate may well be explained by a more efficient countercurrent exchange due to a reduced linear flow rate

The results obtained in this study during *electrical stimulation of the regional sympathetic nerves* agree with the vascular reactions seen in the intestinal mucosa during similar experimental conditions (Svanvik 1973) Thus during steady state of vasoconstriction plasma (blood) flow in the villi is increased slightly above prestimulatory control and a larger portion of intestinal plasma (blood) flow is diverted to the villi while mean transit time here remains unchanged This re distribution of intramural flow is accompanied by an unaltered rate of absorption of krypton and an increase of the fraction of flow fully equilibrated with the luminal contents An increase of the absorption rate above control during sympathetic stimulation might have been expected from the villous circulatory adjustments How ever the mean transit time in the hairpin vascular loops of the villi that seems to be an important factor determining the absorption rate was not changed Moreover a consistent reduction of blood flow around the crypts as proposed by Svanvik (1973) may also depress the absorption rate since the absorbed [8] Kr is probably distributed within the entire mucosa (*cf* Fig 1)

The fact that no transient increase of venous radioactivity was seen upon cessation of constrictor fibre stimulation is explained by the earlier findings that no post stimulatory hyperemia occures in the villous circulation (Svanvik 1973)

The results described above are for several reasons difficult to compare with earlier observations on the relationship between flow and absorption rate First none of the earlier authors had any detailed knowledge of the mucosal haemo dynamics of their experimental models Second krypton is an inert easily diffusible lipid soluble solute which renders comparisons with actively' absorbed water soluble substance *difficult Third many of the experimental manipulations earlier* reported involve complex integrative readjustments For example bleeding will not only lower perfusion pressure but increases also the vasoconstrictor fibre discharge

Finally it should be pointed out that *in vitro* techniques for studying absorption of easily diffusible solutes will not allow any satisfactory analysis of the absorption kinetics since this is markedly affected by the peculiarities of the villous circulation For such studies *in vivo* method are necessary which take into account the effects of the intestinal countercurrent exchanger on the rate of absorption

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AB Vitrum gene usly upplied s with heparin Thanks are also d e to AB Ph rmacia for its supply of Rheoma rodex® and Macrodex® and to AB Has l who generou ly co ered pa t of the expense for techn al assistance

References

BIBER B O LUNDGREN and J SVANVIK The influenc of blood flow n the rate of abs ption of [8] Kr from the small ntestine of the cat *Acta phy ol s nd* 1973 89 227—238

BIBER B O LUNDGREN L STAGE and J SVANVIK An indicato d lution method for studying intest l haemodynamic n the cat *Acta physi l s and* 1973 8 433 –447

HIDOBRO F R VALDES and M DAVILA Influence of the autonomic nervous system on the intestinal absorption of a hypertonic solution of glucose *J Pharmacol exp Ther* 1948 *92* 336—344

KAMPP M O LUNDGREN and J SJOSTRAND The distribution of intravasculary administered lipid soluble substances in the mucosa and the submucosa of the small intestine of the cat *Acta physiol scand* 1968 *72* 469—480

LEE J S and K M DUNCAN Lymphatic and venous transport of water from rat jejunum a vascular perfusion study *Gastroenterology* 1968 *54* 559—567

LUNDGREN O Studies on blood flow distribution and countercurrent exchange in the small intestine *Acta physiol scand* 1967 Suppl 303

LUNDGREN O and J SVANVIK Uptake of [85]Kr from the lumen of the small intestine to the intestinal blood in the cat *Acta physiol scand* 1968 *74* 20A—21A

LUNDGREN O and J SVANVIK Mucosal haemodynamics in the small intestine of the cat during reduced perfusion pressure *Acta physiol scand* 1973 *88* 551—563

NELSON R A and R J BEARGIE Relationship between sodium and glucose transport in canine jejunum *Amer J Physiol* 1965 *208* 375—379

PIIPER J R E CANFIELD and H RAHN Absorption of various inert gases from subcutaneous gas pockets in rats *J appl Physiol* 1962 *17* 268—274

SHIELDS R and C F CODE Effect of increased portal pressure on sorption of water and sodium from the ileum of dogs *Amer J Physiol* 1961 *200* 775—780

SVANVIK J Mucosal haemodynamics in the small intestine of the cat during regional sympathetic vasoconstrictor activation *Acta physiol scand* 1973 *89* 19—29

VARRO V G BLAHO L CSERNAY I JUNG and F SZARVAS Effect of decreased local circulation on the absorptive capacity of a small intestine loop in the dog *Amer J dig Dis* 1965 *10* 170—177

VARRO V I JUNG F SZARVAS L CSERNAY G SAVAY and J ÖKROS The effect of vasoactive substances on the circulation and glucose absorption of an isolated jejunal loop in the dog *Amer J dig Dis* 1967 *12* 46—59

WINNE D Der Einfluss einiger Pharmaka auf die Darmdurchblutung und die Resorption tritiummarkierten Wassers aus dem Dunndarm der Ratte *Naunyn-Schmiedebergs Arch exp Path Pharmak* 1966 *254* 199—224

WINNE D Durchblutung und enterale Resorption *Z Gastroenterologie* 1971 *6* 429—441

Acta physiol scand 1973 89 249—259

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Factors Determining Stroke Volume during Intracoronary Administration of Calcium-Gluconate and Isoproterenol

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Abstract

Bugge Asperheim B *Factors determining stroke volume during intracoronary administration of calcium gluconate and isoproterenol* Acta physiol scand 1973 *89* 249—259

The stroke volume (SV) response to inotropic stimulation of the heart has been studied in open-chest dogs after implantation of an aortic flowmeter pressure recording equipment and piezo-electric ceramics for measuring myocardial chord length (MCL) in the left ventricle Following rapid injections of 1 μg isoproterenol or 100 mg calcium gluconate into the left coronary artery myocardial contractility rose markedly as evidenced by increases of 50—70 % *in the rate of rise of left ventricular pressure (dP/dt)* and aortic flow *(dF/dt)* The SV response was dependent upon left ventricular filling At small ventricular volumes induced by reducing venous return SV remained unchanged during inotropic stimulation In dilated ventricles similar stimulation effected marked increases in SV There was little difference in the increase in SV whether the left ventricle was dilated by increasing preload or afterload End diastolic MCL decreased regularly during inotropic stimulation but the decrease was smaller in a large than in a small ventricle On the other hand myocardial shortening (end-diastolic MCL minus end systolic MCL) increased less in a small ventricle It is concluded that the SV response to inotropic stimulation of the heart is primarily dependent on the end-diastolic dimension of the left ventricle

Cardiac stroke volume (SV) may be varied by altering end diastolic myocardial fibre length or systolic fibre shortening Inotropic stimulation of the heart increases velocity and force of contraction and augments fibre shortening but leads to a reduction in end diastolic myocardial fibre length (Sarnoff and Mitchell 1962 Rushmer 1970) In previous studies of inotropic stimulation of the isolated or intact heart achieved either by electrical stimulation of the heart or by intracoronary injections of inotropic substances increased (Henderson and Barringer *1913* Kelso and Randall 1959 Sarnoff *et al* 1960 Rushmer 1962 Pace *et al* 1969) unchanged (Daggett and Weisfeldt 1965 Noble Trenchard and Guz 1966) or reduced SV (Noble *et al* 1966) have all been demonstrated Since unchanged SV has mainly been observed in conscious dogs it has been postulated that unchanged SV is the normal response to inotropic stimulation and that altered SV is a response due to heart failure in anesthetized animals or to inadequate inotropic stimulation or unsatisfactory recording technique (Noble *et al* 1966 Braunwald 1971)

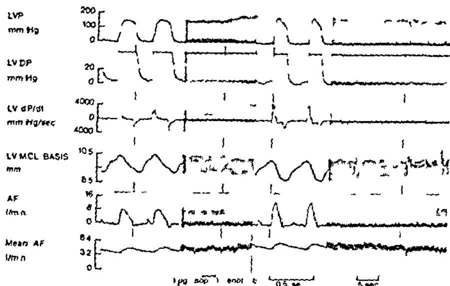


Fig 1 Effects of 1 μg isoproterenol injected into the left coronary artery Stroke volume rose from 23 5 ml to 27 0 ml LVP = left ventricular pressure LVDP = diastolic LVP LV dP/dt = maximum rate of rise of LVP LV MCL = myocardial chord length measured near the base of the anterior part of the left ventricle AF = aortic flow Open chest dog 20 kg b w

The effects of inotropic stimulation of the normal functioning heart however might depend on the size of the heart and consequently on end diastolic myocardial fibre length before stimulation This study was undertaken to characterize the response of the intact heart to inotropic stimulation at different myocardial fibre lengths Large variations in ventricular dimensions were induced by altering either preload or afterload To avoid general effects inotropic stimulation of the heart was effected by rapidly injecting isoproterenol or calcium gluconate into the left coronary artery

Material and Methods

Mongrel dogs of both sexes (18— 4 kg) were anesthetized with sodium ... An initial dose of 25 mg/kg b w was injected i v followed by maint ... to ensure a constant level of anesthesia The dogs were ventilated through a cuffed end tracheal tube with a Cyclator Mk II positive pressure ventilator

The heart was exposed through a left sided thoracotomy and suspended in a pericardial cradle Care was taken to avoid obstructing the central veins Atropine (0 5 mg/kg b w) was administered to prevent reflex bradycardia

Hemodynamic measurements The ... and mean aortic blood flows were recorded by means of a square wave electromagnetic flowmeter (Nycotron Oslo) The term stroke volume (SV) here refers to blood flow into the ascending aorta per beat coronary flow not being taken into account SV was determined by planimetry of the area under the flow curve The linear part of the ascending flow curve was used for calculating the first derivative of aortic flow (dF/dt) and expressed in litres/s

A polyethylene tubing was inserted either through the apex of the left myocardium or through the left atrium for measurements of left ventricular pressure (LVP) and its derivatives dP/dt and $\frac{dP/dt}{IP}$ (where IP is the pressure in the isovolumic period when maximal dP/dt is reached) (Mason Spann and Zelis 1970) Tubes were tied into a femoral or brachial artery

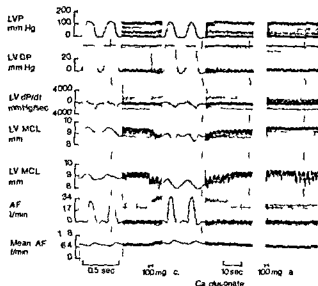


Fig 2 Effects of 100 mg Ca gluconate injected into the left coronary artery Stroke volume ro e from 34 1 ml to 42 ? ml LV MCL = myocardial chord length measured near the base (upper tracing) and near the apex (lower tracing) Right I a injection of 100 mg Ca gluconate had no effect Abbreviations as in Fig 1 Open chest dog 21 kg b v

fo aortic blood pressure recordings Pressures v ere measured with Statham pressure trans du ers P23Gb Calibration of flo v and pre sure eq ipment was performed as previously de scribed (Bugg Asperheim and I ul 1969)

Left ventri ular distances ere record d with pairs of ultrasonic piezo-electric ceramics of lead zi co ate titanate (0 5 × 1 × 3 mm) which together v ith their leads v ere sev n nto the myocardium 8—12 mm apart and parallel to its surface Two pairs of elements ere pla ed in the myocardium at a depth of 3—6 mm near the base of the anterior part of the left ventricle and in the apical region One element acted as tran mitter of ultrasound pul es at a rate of 1000 Hz and the other as receiver The distance bet een the elements is referred to as myo cardial chord length (MCL) The techn que has been published elsewhe e v ith a description of the relationship bet een hanges in ventricul r olume and MCL (Bugge Asperheim Leraand and Kiil 1969 Lekven Bugge Asperhe m and Kiil 1972) All recordings ere made on a Sanborn 8 char nel direct writing oscillograph

Catheteri ation of the left co onary a te y Cath terization was performed according to the technique of Herd and Barger (1964) Th proximal part of the left co onary artery v as dissect d free a polyethylene tub ig PI 240 w ntroduced into the i cumflex b anch and advanced to the bifurcation of th l ft coronary artery The injectate thus reached both branches of the left coronary artery Th s ertfi d by the occu rence of imultaneous respons of MCL at the ba e and ne the ape of the ve tricle

Cont ol obse vat on I 8 dog 1 g isoproterenol o 100 mg calcium gluco ate (Calciu n Sandoz 10 ᶜ) in 1 ml as rap dly i je ted into the left o onary art ry (i c) The e tandard doses were selected in e th ev nduced m ked and al nost im lar instai taneous change in cardiac hemodynam s C respond ng do of sop oter ol or calcium gluconat we e i je ted into the distal part f the ascend ng aorta (i a) These do had l ttle o no eff ct on art al pressure and acted control for the c nje t on confu o i bet een periph ral as ular fects and the c rd effect of the nject on wa thus o ded Furt rmo in 3 d g ap pearance tim of the orona nje tate we e m asur d polar graph ally th a platin m electrode mo nt d on the tip of a adiopaque cathet r n erted into the coronary n s o the right atrium (Bugge A pe heim and Kj k l u 1970) As o b arid was i d as t ac ub stance 1—5 ml b ng nje t d rapidl into the left o onary art n ord to establ sh th max mum ti pas ing belo e pos ble peripheral effects of the dr gs t sted might be expect d

Alterat o i p el ad Afte control observat on n 8 pe im nts n 4 dog en us ret n was decreased b nflating a ballo n mo nted on cardia theter n the inferior ca al e n E ther cal n gl onate o isop oterenol was then i j ted Following d flation of the balloon nou retu n was incr as d by i infusio of 0 9 al e 6 de tı n or blood at 20 ml pe min I e inje tions of alcium-gluconate o isoprot en l we rep ated when marked

TABLE I Effects of intracoronary (i c) injections of isoproterenol (1 μg) and calcium gluconate (100 mg) in control conditions Mean±S E in 8 dogs (mean b w 24 kg)

	n	LVSP mm Hg	LVEDP mm Hg	dP/dt mm Hg 10⁻/sec	$\overline{AP}$ mm Hg	HR beats/min	Aortic Flow Peak l/min
Control	11	113	3 0	2 60	104	157	14 0
±S E		7 7	0 4	0 19	9 4	9 2	2 4
1 μg Isoprot		*126*	*1 9*	*3 40*	113	160	19 6
±S E		9 5	0 5	0 38	9 2	9 4	3 3
p <		0 05	0 005	0 005	ns	ns	0 001
Control	12	121	3 0	2 00	107	158	13 7
±S E		5 5	0 3	0 17	6 1	8 6	2 3
100 mg Ca gluc		*125*	*1 4*	*2 90*	107	158	*17 0*
±S E		5 7	0 4	0 22	5 4	8 1	2 6
p <		0 05	0 001	0 005	ns	ns	0 001

changes in myocardial dimensions had occurred and when left ventricular end diastolic pressure approached 10—12 mm Hg

Alterations in afterload In 8 expts in 4 dogs after control observations with i c injections of calcium gluconate and isoproterenol aortic blood pressure was increased approximately 50 mm Hg by constricting the descending aorta I c injections of the two agents were then repeated

In evaluating differences probability values were obtained with Student s t test for paired data

Results

Control experiments During i c injections of 1 μg isoproterenol and 100 mg calcium gluconate similar cardiac effects were observed Left ventricular dP/dt peak aortic flow and dF/dt increased while end diastolic pressure (LVEDP) declined Left ventricular end diastolic and end systolic myocardial chord length (MCL) decreased *but the difference between them—myocardial shortening (MS)—rose* On an average stroke volume (SV) rose from 19 2 ml to 21 8 ml ($p < 0.05$) (Table I) Fig 1 shows an excerpt from an experiment with i c injection of *1 μg isoproterenol* and Fig 2 the effects of i c injection of 100 mg calcium gluconate The *initial effects* appeared 2—4 s after completion of i c injection of 1 μg isoproterenol When calcium gluconate was injected myocardial changes were already apparent before completion of the injection Maximal effects of both agents were observed 4—9 s *after the injection was completed*

These cardiac effects were not the consequence of recirculation of the i c injected drugs for the following reasons firstly Fig 2 (right) shows that no change occurred following i a injection of 100 mg calcium gluconate into the distal part of the ascending aorta Similarly no effects were observed following *i a injection of 1 μg* isoproterenol except in 2 expts in which aortic blood pressure *declined between* 5 and 10 mm Hg Secondly less time elapsed between the occurrence *of maximal ef*

SV	dF/dt	BASE			APEX		
		EDMCL	ESMCL	MS	EDMCL	ESMCL	MS
ml	l/sec	mm	mm	mm	mm	mm	mm
19.3	9.5	10.9	9.9	1.0	10.1	9.4	0.7
2.5	1.2	0.6	0.5	0.1	1.1	1.1	0.2
22.3	15.1	10.5	9.4	1.1	9.9	9.0	0.9
3.5	2.5	0.6	0.6	0.4	0.1	0.1	0.1
0.05	0.005	0.02	0.005	0.005	ns	0.005	ns
19.[illegible]	9.3	11.0	10.1	0.9	9.5	8.8	0.7
1.7	0.9	0.4	0.4	0.1	0.7	0.7	0.1
21.5	12.3	10.7	9.6	1.1	9.4	8.5	0.9
2.2	1.1	0.4	0.4	0.1	0.7	0.7	0.2
0.005	0.001	0.001	0.01	0.005	ns	0.01	0.005

n number of experiments LVSP left ventricular systolic pressure LVEDP left ventricular end diastolic pressure dP/dt maximum rate of rise of left ventricular pressure $\overline{AP}$ mean aortic pressure HR heart rate SV stroke volume dF/dt maximum rate of rise of aortic flow EDMCL, ESMCL end-diastolic end systolic myocardial chord lengths MS myocardial shortening ns not significant ($p > 0.05$)

fects of inotropic stimulation and the first appearance of recirculation of ascorbic acid recorded in the coronary sinus (8 s as opposed to 12 s in the 3 dogs investigated) Thirdly maximal effects were obtained before any measurable change in heart rate occurred

Alterations in preload Inflation of the balloon in the caval vein reduced aortic blood pressure LVEDP and myocardial dimensions SV fell from 20 to 13.5 ml (Table II)

At this stage i c injections of 1 μg isoproterenol and 100 mg calciumgluconate increased dP/dt $\frac{dP/dt}{IP}$ peak aortic flow and dF/dt ($p < 0.05$) End diastolic MCL declined markedly and MS rose but SV remained unchanged (Table II) Similar alterations were induced by both agents and have therefore been grouped together In the experiment shown in Fig 3 isoproterenol in doses of 0.3 μg and 1.0 μg i c elicited only slight changes in SV After deflation of the balloon venous return was increased by saline/dextran solution or by blood transfusion until LVEDP rose to 10—12 mm Hg End diastolic MCL increased by an average of 12.6 % and SV almost doubled Inotropic stimulation then raised SV by an average of 5.3 ml ($p < 0.01$) which was more than in the control experiments (3.3 ml) and in experiments at low venous return (0.1 ml) ($p < 0.01$) This is illustrated in Fig 4 by the relationship between changes in SV and end-diastolic MCL at the three levels of ventricular filling investigated At high ventricular filling the reduction in end-diastolic MCL was less than at low ventricular filling ($p < 0.05$) Conversely myocardial shortening was greater at high than at low venous return ($p < 0.05$)

TABLE II Effects of intracoronary (i c) injections of isoproterenol (1 μg) and calcium gluconate (100 mg) at variable left ventricular levels of filling Mean ± S E of 8 expts in 4 dogs (mean b w 24 kg)

	LVSP mm Hg	LVEDP mm Hg	dP/dt mm Hg 10^{-3}/sec	dP/dt / IP sec^{-1}	$\overline{AI}$ mm Hg
Reduced venous return					
Control	114	2 5	1 70	28 5	104
±S E	3	0 4	0 16	? 8	4
Injection	124	0 6	2 90	49 0	106
±S E	3	0 4	0 30	3 5	10
p <	0 0?5	0 005	0 005	0 001	ns
Control observations					
Control	139	4 8	2 28	38 9	123
±S E	6	0 2	0 23	6 9	8
Injection	153	3 2	4 40	70 8	1?3
±S E	9	0 7	0 55	10 7	8
p <	0 025	0 02	0 005	0 01	ns
Increased venous return					
Control	151	11 5	? 50	37 1	130
±S E	4	3 0	0 22	3 5	6
Injection	163	6 8	4 30	62 8	128
±S E	3	? 1	0 38	6 7	6
p <	0 01	0 0?5	0 005	0 001	ns

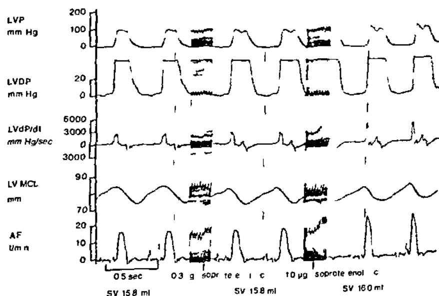


Fig 3 Effects of intracoronary injections of increasing amounts of isoproterenol at reduced venous return Stroke volume remained unchanged Abbreviations as in Fig 1 Open-chest d g 18 kg b w

HR beats/min	Aortic Flow Peak l/min	SV ml	dF/dt l/sec²	BASE EDMCL mm	ESMCL mm	MS mm
172	10 5	13 5	5 9	10 2	9 6	0 6
5	0 6	0 8	0 3	0 1	0 2	0 1
174	14 7	13 6	11 9	9 7	8 9	0 8
6	0 9	1.2	1 1	0 1	0 2	0 2
ns	0 001	ns	0 005	0 001	0 001	0 02
166	15 0	20 0	10 0	10 9	10 1	0 8
8	0 7	1 4	0 4	0 3	0 2	0 1
171	20 0	23 3	16 3	10 4	9 3	1 1
8	1 6	1 9	1 5	0 2	0 2	0 1
ns	0 02	0 02	0 01	0 05	0 025	0 05
174	18 0	26 2	9 4	11 5	10 6	0 9
11	1 0	2 5	0 8	0 2	0 4	0 1
175	25 0	31 5	16 8	11 2	9 9	1 3
5	1 4	2 6	1 3	0 2	0 3	0 1
ns	*0 001*	*0 01*	*0 001*	*0 001*	*0 001*	*0 02*

IP = isovolumic p essure at dP/dt Other abbreviat ons as in Table I

TABLE III Effects of intracoronary (i c) inject ons of isop oterenol (1 μg) and calc um gluconate (100 mg) at cont ol and el vated presst re Mean ±S E of 8 expts in 4 dogs (mean b w 27 kg)

	LVSP mm Hg	LVEDP mm Hg	dP/dt mm Hg 10⁻ / sec	dP dt / IP sec	$\overline{AP}$ mm Hg	HR b ats / m n	Aortic Flo v P ak l m n	SV ml	BASE EDMCL mm	ESMCL m n	MS mm
Control	104	2 9	1 40	25 3	91	149	8 5	14 9	10 6	9 8	0 8
±S E	7	0 3	0 16	0 6	8	6	0 7	0 9	*0 5*	*0 6*	*0 1*
Inj ct on	112	1 0	2 60	44 4	9	153	11 6	14 6	10 3	9 4	0 9
±S E	8	0 4	0 17	1	8	4	1 0	1 2	0 6	0 6	0 1
P <	0 01	0 001	0 02	0 001	ns	n	0 01	ns	0 01	0 01	ns
Incr a ed Pres e											
Control	161	4 7	2 01	28 5	149	141	8 1	11 6	11 2	10	0 7
±S E	3	0 1	0 05	2	5	4	0 6	1 0	0 7	0 7	0
Inject on	168	3 3	3 50	39 0	148	141	13 0	14 5	11 1	10 0	1 1
±S E	9	0 7	0 34	1 6	6	5	0 8	0 8	0 8	0 8	0 1
p <	n	s	0 02	0 01	ns	ns	0 01	0 001	ns	0 05	0 005

… Table I and II

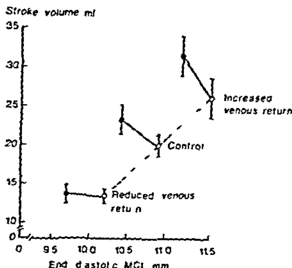


Fig 4 Effects of inotropic stimulation on the relationship between stroke volume and end-diastolic myocardial chord length (MCL) at reduced control or increased venous return. Mean values ± S E of 8 expts in 4 dogs presented in Table II

At the different degrees of ventricular filling investigated there was no significant change in the contractile state of the myocardium before inotropic stimulation as measured by the changes in $\frac{dP/dt}{IP}$

Alterations in afterload A rise in mean aortic blood pressure of 38 mm Hg increased end diastolic MCL ($p < 0.01$) but SV declined ($p < 0.01$) (Table III) Following i.c injection of 1 µg isoproterenol or 100 mg calcium gluconate SV rose ... after elevation of aortic pressure (2 9 ml) than during similar inotropic stimulation at control pressure level (0 4 ml) ($p < 0.01$) Thus enhanced SV response was associated with similar changes in myocardial dimensions as noted by alterations in preload Inotropic stimulation of the heart resulted in a smaller decline in EDMCL and a larger increase in MS in expanded than in unexpanded ventricles

Discussion

Good correlation has previously been demonstrated between changes in left ventricular dimensions and volumes (Lekven *et al* 1972) The observed changes in end diastolic and end systolic MCL therefore represent corresponding alterations in left ventricular volumes In the present study inotropic stimulation of the heart invariably reduced end-diastolic and end systolic MCL The extent of reduction in end diastolic MCL was dependent on left ventricular filling while the extent of reduction in end systolic MCL was not Thus at large ventricular volumes a smaller reduction in end-diastolic MCL occurred during inotropic stimulation than in small ventricles while end-systolic MCL was similarly reduced in the two condition Myocardial shortening i.e the difference between end-diastolic and end systolic MCL always increased during inotropic stimulation However since the reduction in end-diastolic MCL during inotropic stimulation was modest in large ventricles the increase in

myocardial shortening was most marked in this situation Stroke volume therefore rose during inotropic stimulation of the large ventricle whereas it might even fall in small ventricles where the rise in myocardial shortening did not compensate for the reduction in end diastolic MCL Also when afterload was increased by raising aortic blood pressure it could be shown that the stroke volume response to inotropic stimulation was related to ventricular diastolic dilatation and was almost unaffected by the increased resistance to ejection

The relationship observed between changes in end diastolic volume and stroke volume might clarify some of the disagreements of earlier investigators concerning stroke volume changes during inotropic stimulation of the heart Slightly increased stroke volume during inotropic stimulation—chiefly by electrical stimulation of the left stellate ganglion—has regularly been found in isolated heart preparations (Henderson and Barringer 1913 Kelso and Randall 1959 Sarnoff *et al* 1960) However since it is well known that impending heart failure is a frequent occurrence in isolated heart preparations it is probable that some of these hearts were pathologically dilated before inotropic stimulation weakened by deprivation of oxygen and/or depressed by anesthesia It is therefore not surprising that inotropic stimulation appears to reduce the volume of such hearts to improve their function and to increase their stroke volume Caution should however be exercised in drawing conclusions from such experiments to situations in the live animal

In conscious dogs Noble *et al* (1966) found that stroke volume remained unaltered during intracoronary injections of isoproterenol and calcium gluconate They proposed that stroke volume was primarily influenced by extracardiac factors counteracting the increased force and rate of blood ejection into the aorta during inotropic stimulation They suggested that the higher acceleration of blood into the ascending aorta in early systole during inotropic stimulation was followed by exaggerated deceleration of blood in late systole This would be caused by increased distention of the elastic wall of the aorta creating forces opposing the ejection in late systole and preventing a rise in stroke volume From the results of the present study it is unlikely that such a mechanism is the principal regulator of stroke volume Even after elevation of aortic blood pressure inotropic stimulation of the heart caused a marked increase in the stroke volume response The unchanged stroke volume during inotropic stimulation reported by Noble *et al* (1966) may have been the response of a small heart and therefore representative of only one particular situation

Another suggested explanation for the constancy of stroke volume during inotropic stimulation of the heart is that baroreceptor reflexes should alter the primary responses in such a way that contraction rate would increase whilst the force of contraction and degree of systolic emptying would remain unchanged (Wallace Skinner and Mitchell 1963 Daggett and Weisfeldt 1965 Braunwald 1971) In the present study however any such possible compensatory nervous reflex mechanism must have been overruled by the increase in end diastolic myocardial dimensions It is therefore feasible that an unchanged stroke volume during inotropic stimulation of the heart occurs only in certain situations in which the effects of increased

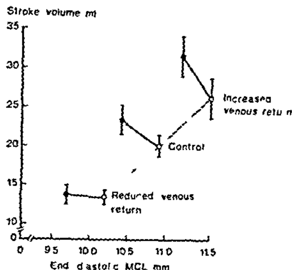


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References

BRAUNWALD E On the difference between the heart s output and its contractile state *Circulation* 1971 *43* 171—174

BUGGE ASPERHEIM B and F KIIL Cardiac response to increased aortic pressure Changes in output and left ventricular pressure pattern at various levels of inotropy *Scand J clin Lab Invest* 1969 *24* 345—360

BUGGE ASPERHEIM B and F KIIL Cardiac mechanisms for regulating stroke volume during elevation of aortic blood pressure in dogs *Scand J clin Lab Invest* 1972 *30* 23—33

BUGGE ASPERHEIM B and J KJEKSHUS Left ventricular pressure and maximum rate of pressure rise as determinants of myocardial oxygen consumption during hemorrhagic hypotension in dogs *Acta physiol scand* 1970 *78* 174—183

BUGGE ASPERHEIM B S LERAAND and F KIIL Local dimensional changes of the myocardium measured by ultrasonic technique *Scand J clin Lab Invest* 1969 *24* 361—371

DAGGETT W M and M L WEISFELDT Influence of sympathetic nervous system on the response of the normal heart to digitalis *Amer J Cardiol* 1965 *16* 394—405

GREGG D E and L C FISHER Blood supply to the heart In *Handbook of Physiology* Vol II Section 2 Circulation Editors W F HAMILTON and P DOW *Amer Physiol Soc* Washington DC 1963 Chapter 44 pp 1517—1584

HENDERSON Y and T B BARRINGER The conditions determining the volume of the arterial blood stream *Amer J Physiol* 1913 *31* 288—299

HERD J A and C BARGER Simplified technique for chronic catheterization of blood vessels *J appl Physiol* 1964 *19* 791—792

KELSO A F and W C RANDALL Ventricular changes associated with sympathetic augmentation of cardiovascular pressure pulses *Amer J Physiol* 1959 *196* 731—734

LEKVEN J B BUGGE ASPERHEIM and F KIIL Instantaneous left ventricular volume determined by ultrasonic measurement of local myocardial distances *Scand J clin Lab Invest* 1972 *29* 5—14

MASON D T J F SPANN JR and R ZELIS Quantification of the contractile state of the intact human heart *Amer J Cardiol* 1970 *26* 248—257

NOBLE M I M D TRENCHARD and A GUZ Left ventricular ejection in conscious dogs II Determinants of stroke volume *Circulat Res* 1966 *19* 148—152

PACE J B W K KEEFE J A ARMOUR and W C RANDALL Influence of sympathetic nerve stimulation on right ventricular outflow tract pressures in anesthetized dogs *Circulat Res* 1969 *24* 397—407

PALEY H W I G McDONALD J BLUMENTHAL and J MAILHOT The effects of posture and isoproterenol on the velocity of left ventricular contraction in man *J clin Invest* 1971 *50* 2283—2294

RUSHMER R F Effects of nerve stimulation and hormones on the heart the role of the heart in general circulatory regulation In *Handbook of Physiology* Vol I Section 2 Circulation Editors W F HAMILTON and P DOW *Amer Physiol Soc* Washington DC 1962 Chapter 16 pp 533—550

RUSHMER R F pp 82—87 In *Cardiovascular Dynamics* W B Saunders Company Philadelphia 1970

SARNOFF S J S K BROCKMANN J P GILMORE R J LINDEN and J H MITCHELL Regulation of ventricular contraction Influence of cardiac sympathetic and vagal stimulation on atrial and ventricular dynamics *Circulat Res* 1960 *8* 1108—1122

SARNOFF S J and J H MITCHELL Control of function of heart In *Handbook of Physiology* Vol I Section 2 Circulation Editors W F HAMILTON and P DOW *Amer Physiol Soc* Washington DC 1962 Chapter 15 pp 489—532

VAN CITTERS R L D L FRANKLIN and R F RUSHMER Left ventricular dynamics in dogs during anesthesia with alpha-chloralose and sodium pentobarbital *Amer J Cardiol* 1964 *13* 349—354

WALLACE A G N S SKINNER and J H MITCHELL Hemodynamic determinants of the maximal rate of rise of left ventricular pressure *Amer J Physiol* 1963 *205* 30—40

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From the Department of Physiology University of Lund Sweden

Effects of Acetylcholine and Papaverine on the Secretion and Blood Flow from the Pancreas of the Cat

By

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Abstract

LENNINGER S *Effects of acetylcholine and papaverine on the secretion and blood flow from the pancreas of the cat* Acta physiol scand 1973 89 260—268

Acetylcholine and papaverine were injected into the coeliac artery of cats in which the pancreatic secretion and blood flow were recorded Acetylcholine evoked a small secretion from the resting gland but papaverine did not. During secretion induced by secretin both acetylcholine and papaverine caused an increase in the rate of secretion and both drugs also increased the rate of blood flow The relationship between the changes in secretion and blood flow is discussed

Electrical stimulation of the vagus nerve and i v injection of parasympathomimetic agents have been shown to stimulate the flow of juice from the pancreatic gland of the cat (Brown Harper and Scratcherd 1967 Lenninger and Ohlin 1971 Lenninger 1971) Results have been presented suggesting that these effects may be caused by a cholinergic action on the secretory cells (Lenninger and Ohlin 1971) It has also been proposed however that the effect of vagal stimulation is not a direct one but mediated by secretin made available to the gland by vasodilatation (Brown *et al* 1967) An important argument for this hypothesis was that vagal excitation was found not to stimulate the secretion unless the gland was already secreting in response to secretin (Brown *et al* 1967) Later investigations have shown however that vagal stimulation can evoke pancreatic secretion from the anesthetized cat also in the absence of exogenous secretin (Lenninger and Ohlin 1971) There are nevertheless other findings suggesting that the secretion caused by cholinergic stimulation in fact is secondary to a blood borne stimulant Thus the vagally evoked secretion is relatively resistant to atropine and the juice has a relatively high content of bicarbonate (Brown *et al* 1967 Lenninger and Ohlin 1971) features that are typical of the secretion caused by secretin It should also be pointed out that vasodilatation in the pancreas of the cat indeed occurs in response to cholinergic stimulation (Holton and Jones 1960 Hilton and Jones 1968 Barlow Greenwell

Harper and Scratcherd 1968) In the present experiments attempts have been made to find out whether a causal relationship exists between pancreatic blood flow and secretory rate in the cat Secretion and blood flow have therefore been simultaneously measured and the effects of arterially injected acetylcholine and papaverine have been studied Acetylcholine was chosen because of its probable role as the natural transmitter of vagal impulses to the gland and papaverine was chosen as a vasodilating agent without known ecretory effects Because the effects of these two agents on the resting pancreas of the cat have not earlier been systematically studied this was first done in a separate eries of experiments

Methods

The experiments were performed on 18 cats 9 cats (mean weight 3 0 kg range 2 6—3 7 kg) were used for purely secretory studies and 9 cats (mean weight 3 1 kg range 2 6—4 2 kg) were used for the study of the relation between secretion and blood flow The cats were fasted over night and anesthetized with ether followed by chlorase i v (80 mg/kg) The stomach was drained by a tube in erted through the oesophagus and the trachea was cannulated The abdomen was opened by a midl ne incision and the pylorus occluded by a l gature The animals used for the secretory studies were prepared as previously described (Lenninger and Ohlin 1971) i e the pancreatic duct was cannulated both at the head and the tail of the gland The animals were then heparinized (1000 i U/kg) A pol ethylene catheter for arterial injections (OPP Portex outer diam 1 15 mm) tapered and bent at its end was introduced through the left femoral artery and pushed up the aorta until the tip of the catheter entered the coeliac artery This position was ascertained by inspecting the root of the artery through the wall of which the catheter could be seen The left femoral vein was cannulated for the infusion of secretin The blood pre sure was measured in the right carotid artery using a mercury manometer

The cats used for the study of the relation between secretion and blood flow were prepared in the fo lowing way Only the duct of the head was cannulated The blood vessels between the pancreas and the stomach and between the pancreas and the oment were severed The sple n was remo ed After heparinization a polyethylene tube (PF 50 outer diameter 0 97 mm) was introduced through the left gastric artery into the coeliac artery to a point about 2 cm distal to the aorta. This catheter was used for arterial injections Another polyeth lene tube (PE 260 inner diameter 1 77 mm) was introduced into a branch of the splenic vein and a ligature was tied round the mai i splenic vein where it joins the portal vein The blood was led via pol ethylene tubings from the cannulated splen c vein to a drop chamber and then returned to the animal through the right external jugular vein

The drops of secretion and blood were reg stered by 2 photoelectric cells which operated 2 ordinate recorders These marked the rate of flow on 2 separate sheets of smoked paper

The prote n content was determined by measuring the optical density of the jui e at 280 mμ in a Zei s spectrophotometer and comparing the results to an albu nin standard

The following drugs ere used Secretin (GIH Research Unit Karolinska Inst tutet Stockholm, Sweden) acetylcholine chloride and papaverine sulphate The secretin was gi en as a continuous injection into the left femoral vein at a rate of 0 03 U/kg min In some experiments it was also given at lower rates (0 02 0 01 and 0 00 U/kg m n) The acetylcholine and papaverine were given in doses of 100 g and 10 mg respecti ely dissol ed in 10 ml of saline The drugs were injected into the coeliac artery from an infu ion pump during period of about 10 min The interval between the doses was normall 10 to 20 min

Results

Secretory studies

No ecretory activity was seen at the time of cannulation After the duct had been flushed with saline a slow and irregular flow emerged from of the 9 cats The amounts seldom exceeded one drop in 10 min

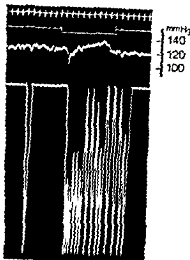


Fig 1

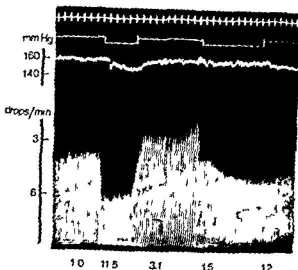


Fig 2

Fig 1 Cat 3 0 kg Chloralose anesthesia *Secretin continuously injected i v at a rate of 0 01 U/kg/min Acetylcholine injected into the coeliac artery in a dose of 100 μg at signal Recordings from above Time in min signal blood pressure drops of pancreatic juice*

Fig 2 Cat ? 6 kg Chloralose anesthesia *Secretin continuously injected i.v at a rate of 0 03 U/kg/min Acetylcholine (100 μg)* and *papaverine (10 mg)* injected into the coeliac artery at first and second signal *respectively Recordings as in Fig 1 Figures below panel denote protein concentration in mg/ml*

Effect of acetylcholine no secretin injected Acetylcholine was given to 8 cats before any secretin was injected *Usually three doses were given to each animal It used a small flow of pancreatic juice in all the cats The responses were irregular however and not all injections caused secretion The mean response was 1 4 drops (range 0—6 drops) In cases where no secretion emerged the duct was flushed during or between the injections of acetylcholine The procedure did not alter the secretory response however Acetylcholine was also injected to these cats after secretin had been given continuously for several hours These injections of acetyl choline were made 20 to 60 min after the injections of secretin were stopped By that time the flow of juice from the glands was of the same order as in the beginning of the experiments The mean secretory response to acetylcholine was now 2 9 drops (range 0—8 drops) This amount is significantly larger ($p < 0.02$) than the amount secreted before the secretin had been given*

The acetylcholine caused the blood pressure to fall 10—20 mm Hg during the first minutes of injection The pressure thereafter returned to pre injection level

Effect of acetylcholine during continuous injection of secretin The injection of secretin in a dose of 0 03 U/kg min caused a secretion from these cats at rates varying between 11 and 48 drops/10 min (mean 25 6 drops S E 6 1 corresponding to 0.51 ml ± 0 12 ml) The rate of secretion increased in all animals during the injection of acetylcholine The increase in rate of flow appeared promptly and was

TABLE I Changes in rate of secretion and blood flow from the pancreatic gland during arterial injection of acetylcholine

	drops/10 min			ml/ 10 min			
	range	mean	SE	mean	range	mean	SE
secretion	−3+46	12 25	8 55	0 74	−23+200	69 07	45 71
blood flow	−8+1034	253 10	194 73	10 1	−11+332	91 89	64.29

usually well maintained during the injection The mean increase was 22 drops/10 min S E 7 5 corresponding to 0 44 ml ± 0 15 ml In 3 cats the rate of injection of secretin was lowered so as to cause no or only a trace of secretion Under these circumstances a relatively brisk secretion (6—14 drops) could be elicited by the injection of acetylcholine (Fig 1) The response was not seen during all the injections of acetylcholine however In some instances the secretion did not start until the duct had been flushed with saline

The protein concentration of the juice was measured when the gland was secreting in response to secretin It was found that the total content of proteins was significantly larger ($p < 0\ 02$) in the juice collected during the injection of acetyl choline than in the juice collected immediately before the injection

Effect of papaverine no secretin injected Papaverine was given to 3 cats before secretin was given It did not cause any secretion neither before nor after flushing the duct with saline Papaverine was also given to 3 cats which had previously secreted to continuously injected secretin Two of these cats secreted 6 and 13 drops respectively during the injection of papaverine 75 and 90 min after the injection of secretin had been interrupted At that time no resting secretion was recorded

A gradual reduction of the blood pressure with 10—20 mm Hg was often seen during the injections of papaverine

Effect of papaverine during continuous injection of secretin Papaverine was given to 8 cats which secreted in response to intravenously injected secretin In all cats an increase in the rate of secretion was recorded The rate increased more gradually as when acetylcholine was injected (Fig 2) and also remained increased about 10 minutes after the interruption of the injection The mean increase in number of drops was 12 1/10 min S E 1 93 corresponding to 0 24 ml ± 0 04 ml In one cat to which secretin was given at a rate of 0 005 U/kg min. without causing any secretion papaverine caused a flow of 38 drops/10 min There was no increase in the output of proteins in response to the injections of papaverine

Simultaneous measurement of pancreatic secretion and blood flow

Nine cats were used for these experiments Secretin was continuously injected into a femoral vein at a rate of 0 03 U/kg min during the whole experiments This caused a pancreatic secretion varying between 4 and 34 drops/10 min (mean 16.3 S E. 5 1

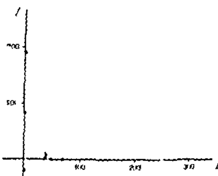


Fig 3 Relation between change in rate of secretion (ordinate) and blood flow (abscissa) in response to arterially injected acetylcholine ($r = 0.18$, $p < 0.05$)

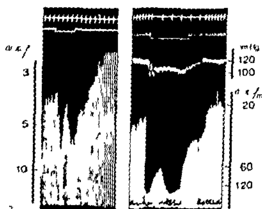


Fig 4

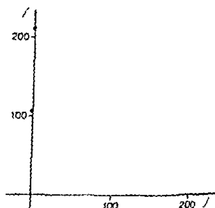


Fig 5

Fig 4 Cat 3.7 kg Chloralose anesthesia Secretin continuously infused i.v. at a rate of 0.03 U/kg/min Acetylcholine injected into the coeliac artery in a dose of 100 µg at signal Simultaneous recordings from above left panel time in min signal rate of secretion right panel time in min signal blood pressure rate of blood flow

Fig 5 Relation between change in rate of secretion (ordinate) and blood flow (abscissa) in response to arterially injected papaverine ($r = 0.21$, n.s.)

corresponding to 0.32 ml ± 0.10 ml) This is significantly less ($p < 0.001$) than in the experiments where only the secretion was measured The blood flow from the cannulated splenic vein varied between 90 and 700 drops/10 min (mean 260 ± 114 corresponding to 10.4 ml ± 4.6 ml)

Effects of acetylcholine on the rate of secretion and blood flow Acetylcholine in doses of 100 µg was injected at times when both the secretion and blood flow were reasonably stable In 7 of the animals an increase in the rate of secretion was regularly seen in one there was no change and in one the rate declined with 20 per cent The changes for the whole group are listed in Table I The mean increase in secretion both totally and percentually was smaller ($p < 0.05$) in these cats as compared with those in which only the secretion was measured

TABLE II Changes in rate of secretion and blood flow from the pancreatic gland during arterial injection of papaverine

	drops/10 min			ml/10 min			
	range	mean	SE	mean	range	mean	SE
secretion	+ 3+22	14.17	4.16	0.29	+50+210	115.0	38.92
blood flow	−60+90	26.0	30.53	1.04	−20+100	27.7	21.82

The pancreatic blood flow increased during most of the injections of acetylcholine (Table I). The flow rate usually increased promptly at the start of the injection and also decreased as soon as the injection was finished. Often the rate was seen to fluctuate considerably during the period of injection (Fig. 4).

The rate of secretion and blood flow varied in the same direction in response to 22 of the 27 injections of acetylcholine and the percentual changes of the secretion and blood flow are positively correlated ($r = 0.48$, $p < 0.05$) (Fig. 3). In many of the recordings a parallelism in the two parameters could be seen (Fig. 4).

Acetylcholine was injected to 4 of the cats after atropine in a dose of 0.1 mg/kg had been given i.v. In one animal a slight increase (14 per cent) in the rate of secretion occurred, otherwise the effects of acetylcholine were annulled. The blood pressure was not changed during these injections.

Effects of papaverine on the rate of secretion and blood flow. Papaverine in doses of 10 mg was injected arterially to the 9 cats in which the rates of secretion and blood flow were simultaneously recorded. In all the animals the rate of secretion increased during the injections and the blood flow usually increased (Table II). The increase in rate of secretion was similar to that seen in the experiments where only the secretion was measured. The changes in blood flow and secretion appeared gradually and the effects often lasted 5—10 min. after the injection was completed. The changes of the two recordings often followed each other quite closely during the injections. The correlation between the changes was not found to be statistically significant, however (Fig. 5).

Discussion

The studies of the secretory effects of acetylcholine and papaverine and the effects of these two agents on the relation between secretion and blood flow required different preparations and were therefore carried out separately. Earlier studies on the effect of cholinergic stimulation on the pancreatic secretion have demonstrated that particular means such as double cannulation of the duct and rinsing the duct with saline often is of great help in order to detect the secretory response of the gland (Lenninger and Ohlin 1971). It is, on the other hand, desirable to operate as little as possible on the pancreas when the secretion is to be studied and interference with the blood supply of the gland should therefore be avoided. The present results show in fact that the gland secretes at a lower rate when preparations are made to measure both secretion and blood flow.

The blood flow was measured with a direct method which has the advantage of giving results that are relatively easy to interpret A drawback with the method is that a certain interference with the blood flow can hardly be avoided This was probably the cause of the lower rate of secretion in the blood flow experiments It can on the other hand be calculated that the rate of blood flow in these experiments was of the same order as that obtained by other investigators using other methods i e 25—80 ml/100 g min (Delaney and Grim 1966 Eliassen Folkow and Hilton 1972) Thus a mean flow of 10 4 ml/10 min was recorded from the splenic vein This blood emerges from the tail of the gland with an approximal weight of 2 g and the calculated flow will then be approximately 50 ml/100 g min

For technical reasons the blood flow was measured from only the tail of the gland while the secretion was drained from the whole gland The drugs which were injected into the coeliac artery can be assumed to have reached both the head and the tail of the gland Thus the arterial injection of acetylcholine increased the venous outflow with an average of 100 per cent and of papaverine with an average of about 30 per cent Truly these values are below the maximal dilatation mentioned to occur in response to these agents (Hilton and Jones 1968 Eliassen *et al* 1972) but it should be pointed out that during the injections the blood flow was for brief periods increased several hundred per cent also in the present experiments The possibility has to be considered however that the drugs by chance be diverted mainly to the head of the gland In such a situation only an effect on the secretion can be expected to be seen Such observations were in fact occasionally made

The present experiments have shown that acetylcholine causes a secretory response the resting pancreas of the cat The response is small however and smaller than the secretion evoked by vagal stimulation (Lenninger and Ohlin 1971) The response to acetylcholine after a period of secretion caused by secretin was still small although increased in relation to the response before the secretin was given. This shows that arterially injected acetylcholine is a weak stimulant of pancreatic secretion in the cat also when optimal conditions for the flow of juice have been created

Larger doses of acetylcholine might have been able to cause a larger response in some cats but were not regularly tried because of the general effects of the drugs then encountered Furthermore the dose of 100 µg/10 min of acetylcholine was found to give a considerable increase in the rate of secretion when secretin was injected and was also large enough to cause an increase in the output of proteins from the gland

The increase in secreted volume of the secretin stimulated gland was more than 10 times as large as the secretion produced by the same dose of acetylcholine given to the resting gland The potentiating effect of secretin on the response to acetylcholine was particularly evident in the experiments where subthreshold doses of secretin were given This effect may be due to a synergistic action of the two agents on the secretory cells The large secretory response of the combined stimuli may also be caused by vascular changes however This later hypothesis is supported by

the results of the injections of papaverine and by the experiments in which both secretion and blood flow were measured Acetylcholine caused an increase in the rate of blood flow that was correlated to the increase in secretion This applies to the whole group of observations of simultaneous changes in blood flow and secretion but often also to the changes during individual period of injection Although this does not proof that these two variables are causally related the findings are well compatible with such a hypothesis

A causal relationship between blood flow and secretion is also indicated by some of the effects of papaverine This substance had no secretory effect before secretin was given but caused quite a large increase in the secretion during the injection of secretin The finding that papaverine caused secretion in two cats more than an hour after the injection of secretin was interrupted might be taken as an indication of a genuine secretory effect of this drug a more probable explanation is however that subthreshold concentrations of secretin were present in the blood in these experiments and that the effect was caused by the combined actions of papaverine and secretin

A significant correlation between the changes in secretion and blood flow should be expected in case one effect be dependent on the other This was not found when papaverine was injected however Furthermore the secretion increased relatively more than the blood flow during these injections This lack of covariation is possibly due to an uneven distribution of the drug to the head and the tail of the gland or to different responses of the vessels in the two parts of the gland

The present experiments thus seem to indicate that vasodilatation in the pancreas may cause an increase in the rate of secretion when secretin is available to the gland The increase seen during cholinergic stimulation may therefore at least partly be due to vasodilatation

The way by which vasodilatation increases the secretion is not clear Barlow (1927) who found that sodium nitrite increased the secretory rate of the pancreas of the dog has proposed that the vasodilatation improves the nutritional conditions of the gland It has also been suggested that the vasodilatation increases the rate of secretion by facilitating the supply of secretin to the gland (Brown *et al* 1967) This must imply then that a gradient exists in the concentration of secretin between the secretory cell and the blood either due to a deficient blood flow during the periods when only secretin is given or to a rapid turnover of secretin in the secretory cells To discuss this hypothesis more knowledge of the distribution and metabolism of secretin than is available today is required

References

BARLOW O W The influence of sodium nitrite peptone and pilocarpine on the external secretion of the pancreas *Amer J Physiol* 1927, *81* 189—196

BARLOW T E J R GREENWELL A A HARPER and T SCRATCHERD Factors influencing pancreatic blood flow In *Blood Flow through Organs and Tissue* Ed W H Bain and A M Harper E & S Livingstone LTD Edinburgh 1968 pp 469—484

BROWN J C A A HARPER and T SCRATCHERD Potentiation of secretin stimulation of the pancreas *J Physiol* (Lond) 1967 *190* 519—530
DELANEY J P and E GRIM Influence of hormones and drugs on canine pancreatic blood flow *Amer J Physiol* 1966 *211* 1398—1402
ELIASSEN E B FOLKOW and S HILTON Blood flow and capillary filtration capacities in salivary and pancreatic glands as compared with skeletal muscle In Abstracts from Meeting of the Scandinavian Physiological Society in Uppsala 24—25 November 1972 *Acta physiol scand* 1973 *87* 11A
HILTON S M and MURIEL JONES The role of plasma kinin in functional vasodilatation in the pancreas *J Physiol* (Lond) 1968 *195* 521—533
HOLTON PAMELA and MURIEL JONES Some observations on changes in the blood content of the cat s pancreas during activity *J Physiol* (Lond) 1960 *150* 479—488
LENNINGER S Effects of parasympathomimetic agents and vagal stimulation on the flow in the pancreatic duct of the cat *Acta physiol scand* 1971 *82* 345—353
LENNINGER S and P OHLIN The flow of juice from the pancreatic gland of the cat in response to vagal stimulation *J Physiol* (Lond) 1971 *216* 303—318

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The Effect of Hemorrhage on Hepatosplanchnic Hemodynamics, Liver Function and Hepatic Metabolism

By

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Abstract

KRARUP N *The effect of hemorrhage on hepatosplanchnic hemodynamics liver function and hepatic metabolism* Acta physiol scand 1973 89 269—277

Cats in the postabsorptive state and anesthetized with chloralose were used for the experiments Following a control period the cats were bled approximately 25 % of the blood volume The total liver blood flow decreased in parallel to the mean arterial blood pressure due to a large decrease in portal flow whereas the hepatic arterial flow remained constant Thus the splanchnic vascular resistance was not changed the hepatic arterial resistance decreased and the gastrointestinal resistance increased after the hemorrhage Also the portal venous resistance was significantly increased The hemorrhage resulted in hepatic glycogenolysis and peripheral glycolysis but neither the splanchnic elimination of ethanol consumption of oxygen nor hepatic dye elimination was decreased and the hepatic redox level remained unaltered The results indicate that although hemorrhage is accompanied by marked changes in hepatosplanchnic hemodynamics the intrahepatic distribution of blood flow is not subject to gross alterations

Hemodynamic studies of the hepatic circulation have revealed that the intrahepatic portal resistance increases after hemorrhage (Friedman Frank and Fine 1951 Muller and Smith 1963 Greenway Lawson and Stark 1967 b) In transillumination studies in rats it was observed that hemorrhage caused a pronounced sinusoidal constriction with disappearance of many of the sinusoids apparent before the bleeding (Seneviratne 1950 Friedman *et al* 1953 Pointner Peschl and Stefenelli 1970 Ho 1972) After hemorrhage in primates the sinusoids became dilated and engorged with slowly moving erytrocytes (Vanecko Szanto and Shoemaker 1969) indicating a redistribution of the intrahepatic blood flow A gross redistribution of the intrahepatic blood flow is likely to be accompanied by a corresponding decrease in the functional capacity of the liver or liver mass

The present experiments were designed to explore whether such a redistribution occurs by comparing the effect of moderate hemorrhage on the hepatosplanchnic hemodynamics with the effect on the functional capacity of the liver The functional capacity was determined by the splanchnic elimination of ethanol and of Indocyanine Green (ICG) as well as by estimations of the splanchnic oxygen consumption and lactate pyruvate and glucose metabolism

Methods

15 cats weighing 2.6—3.6 kg were fasted overnight and anesthetized with chloralose (50 mg/kg) after an initial dose of Nembutal (30 mg). The abdomen was opened through a midline incision and a catheter was manually guided into a liver lobe, usually the left, via the right external jugular vein and right heart. The position of the catheter was controlled at the end of the experiments. A femoral vein was used for infusions of ethanol and ICG. After a recovery and equilibration period of 90 minutes after the operation, blood samples were drawn every 15 minutes from a femoral artery and the hepatic vein. Following a control period of 75 min the cats were bled 15 ml/kg by withdrawing 3 portions of blood, each 5 ml/kg. The first two portions were drawn within 5 min and the last 15—20 min thereafter. In this way the hemodynamic parameters measured reached a new steady state immediately after drawing the last portion.

Mean arterial blood pressure was recorded from a catheter placed in a femoral artery by means of a condensator manometer. Furthermore, in 8 of the cats portal venous flow and pressure was recorded by means of an electromagnetic flowmeter (Nycotron, Oslo) and a condensator manometer. The portal vein was exposed and cleaned and a flowprobe (I.D. 3.0 mm) was placed around it. Zero adjustment of the flowmeter was performed during the experiments according to the factory's manual, and calibration of the flow probe was made periodically on the abdominal aorta. A polyethylene catheter (I.D. 0.40 mm) was introduced into the portal vein via a peripheral mesenteric vein for pressure measurement. In these experiments hepatic venous pressure was recorded intermittently from the hepatic venous catheter. The output from the flowmeter and the manometers were recorded on a Beckman S II Dynograph.

The body temperature was kept at 38.5° C by gentle heating.

Administration of ethanol and indocyanine green

Ethanol was infused continuously (36 μmol/kg/min) after a priming dose (6.5 mmol/kg) resulting in blood concentrations of ethanol at which the hepatic elimination capacity of ethanol is saturated. This means that the elimination rate of ethanol within wide limits is independent of the hepatic blood flow and the arterial blood concentration (Larsen 1963) and therefore reflects the functional liver mass. ICG (Hynson, Westcott and Dunning) was given as a priming dose (300 μg/kg) followed by a continuous infusion of 5 μg/kg/min which resulted in an almost horizontal time concentration relationship of ICG in the plasma. The priming doses of ethanol and ICG were dissolved in isotonic NaCl 20 ml, and for continuous infusions ethanol and ICG were dissolved in isotonic NaCl 50 ml, of which 0.1 ml/min was infused. To increase the stability of the dye, albumin was added and the infusion was protected against light.

Analytical procedure

Ethanol and plasma ICG was determined as previously described (Krarup and Larsen 1972). Hemoglobin was determined photometrically, and the oxygen saturation as described by Siggaard Andersen, Jørgensen and Næraa (1962) with the modification previously described (Krarup 1973). Blood glucose was determined enzymatically be means of glucoseoxidase (GLOX, KABI) and lactate and pyruvate fluorometrically as described by Olsen (1971).

Calculations

The total elimination rate of ethanol was calculated as the amount of ethanol infused per minute corrected for the amount retained in or disappeared from the solvent space which was set to 65 % of the body weight according to Larsen (1963). The splanchnic elimination rate of ethanol, consumption of oxygen and uptake/output of lactate, pyruvate and glucose was calculated by multiplying the respective arterio-hepatic venous difference by the estimated hepatic blood flow (EHBF). The latter was determined by means of ICG using the Fick principle. The oxygen concentration in arterial and hepatic venous blood were calculated from the saturation per cent and the hemoglobin concentration, assuming an oxygen binding capacity of 1.34 ml/g hemoglobin.

Liver function was also followed by determining the plasma clearance and extraction ratio of ICG, which is exclusively eliminated by the liver. Clearance was calculated as the amount of ICG eliminated per minute divided by the arterial plasma concentration. The elimination rate of ICG was estimated as the infusion rate corrected for the amount retained in the plasma volume set to 5 % of the body weight. The extraction ratio of ICG was calculated as the arterio-hepatic venous ICG difference divided by the arterial concentration.

Application of a flow probe on the hepatic artery would interrupt most of the hepatic nerve supply. The hepatic arterial flow was therefore not measured electromagnetically but calculated as the difference between the EHBF and the portal flow determined electromagnetically. The resistance in the hepatosplanchnic vascular bed was calculated in peripheral resistance units in the following manner

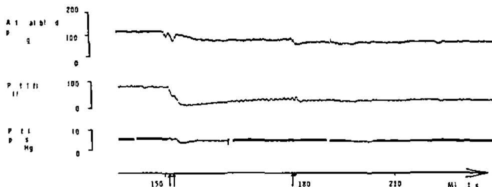


Fig 1 The effect of hemorrhage on mean arterial blood pressure and portal venous blood flo and pressure Each arrow indicates withdrawal of blood (5 ml/kg b wt)

$$SVR = \frac{BP}{EHBF}$$

$$HAR = \frac{BP}{HAF}$$

$$GIR = \frac{(BP-PP)}{GIF}$$

$$PVR = \frac{(PP-VP)}{GIF}$$

(SVR splanchnic vascular resistance HAR hepatic arterial resistance GIR gastrointestinal vascular resistance PVR portal venous resistance mmHg PP portal venous pressure mmHg BP mean arterial blood pressure mmHg VP hepatic venous pressure mmHg HAF hepatic arterial flow ml/min GIF gastrointestinal (= portal venous) flow ml/min

Statistical procedures

The effect of hemorrhage was tested by the method of paired comparisons using the Student t test Parameters not normally distributed (arterial fraction of the total liver blood flow vascular resistances and lactate/pyruvate ratios) was tested by the Wilcoxons signed—ranks test Differences between means in group I and II (Table II) was tested by the Student t test.

Results

1 Hepatosplanchnic hemodynamics

In the control period the hemodynamic parameters measured remained essentially constant The first and second withdrawal of blood caused immediate decreases in arterial and portal blood pressure and portal flow but steady levels were not obtained until after the third withdrawal of blood (Fig 1) BP decreased from 120 ± 7 to 88 ± 6 mm Hg which was statistically significant ($p < 0.001$) The decrease in PP (from 6.3 ± 0.6 to 5.6 ± 0.7 mm Hg) and a slight decrease observed in VP (from 1.0 ± 0.8 to 0.5 ± 0.7 mm Hg) were not significant ($p > 0.5$)

The steady state values of flows and resistances before and after hemorrhage are summarized in Fig 2 It appears that EHBF (15 expts) decreased in parallel to

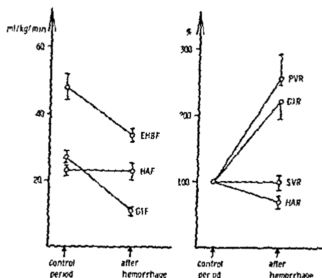


Fig 2 Steady state levels of gastrointestinal (GIF) and total liver blood flow (EHBF) hepatic arterial blood flow (HAF) before and after hemorrhage Furthermore the effect of hemorrhage on total splanchnic (SVR) gastrointestinal (GIR) hepatic arterial (HAR) and portal venous (PVR) resistances are shown The bars indicates S E (EHBF SVR $n = 15$ GIF HAF GIR HAR PVR $n = 8$)

ly the arterial fraction of the total liver flow increased (from 45 ± 7 to 68 ± 11 per cent $p < 0.01$) As EHBF decreased to the same extent as BP no change in SVR was observed In the 8 expts in which portal flow and pressure was measured GIR as well as PVR increased about 100 % after hemorrhage ($p < 0.001$) whereas HAR decrea ed by 25 % ($p < 0.01$)

Liver function

The effects of the hemorrhage on liver function are listed in Table I It is een that there was a slight, not significant increase in the splanchnic as well as total elimina tion rate of ethanol The plasma clearance of ICG was slightly decreased whereas the extraction ratio of the dye increa ed

Judged from the elimination rate of ethanol however it appeared that the hemorrhage resulted in two different responses I) 9 expts in which no effect on ethanol elimination was found and II) 6 expts in which an increase in the total as well as splanchnic elimination rate was found. In Table II the experiments are grouped

TABLE I The effect of hemorrhage (15 ml/kg) on ethanol and ICG elimination (mean and S E in 15 cats The numbers in brackets indicate the total eliminat on of thanol

	Splanchnic elimination of Ethanol µmol/kg/min	Plasma clearance of ICG ml/kg/min	Extraction ratio of ICG per cent
Control period	30 ± 2.0 (35 ± 1.5)	$3.9 \pm 0.?$	13 ± 1.3
After hemorrhage	32 ± 1.8 (38 ± 1.9)	3.5 ± 0.2	16 ± 1.3
Effect of hemorrhage	$p < 0.10$	$p < 0.001$	$p < 0.01$

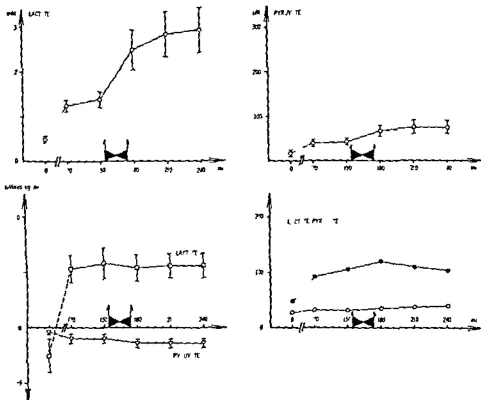


Fig 3 The effect of hemorrhage on the arterial lactate and pyruvate concentrations the splanchnic output (positive)/uptake (negative) of lactate and pyruvate and the arterial (○) and hepatic venous (●) lactate/pyruvate ratios The bars indicates S E n(= 15) The ethanol infusion is started immediately after the sampling at time 0 Withdrawal of blood is performed between the arrows indicated on the abscissa

TABLE II The effect of hemorrhage (15 ml/kg) on splanchnic oxygen extraction and consumption in 9 cats in which the ethanol elimination remained unaltered after the hemorrhage (group I) and in 6 cats in which the ethanol elimination increased (group II)

	Splanchnic elimination of Ethanol μmols/kg/min		Splanchnic oxygen extraction mM		Splanchnic oxygen consumption μmole kg min	
	A	B	A	B	A	B
I	33 ± 2.9	31 ± 2.9	2.5 ± 0.[illegible]	3.3 ± 0.[illegible]	123 ± 9	120 ± 8
			($p < 0.01$)		($p > 0.10$)	
II	25 ± 1.1	33 ± 1.4	2.3 ± 0.6	4.5 ± 0.9	11[illegible] ± 14	148 ± 19
			($p < 0.001$)		($p < 0.01$)	
Difference between I and II	p 0.001	p 0.10	$p > 0.10$	$p < 0.10$	p 0.10	$p > 0.10$

A control period

B after [illegible]

according to the effect of hemorrhage on the rate of ethanol elimination A simultaneous increase in the total and splanchnic ethanol elimination of more than 5 µmol/kg/min was considered significant and the experiment was placed in group II From the table it appears that the ethanol elimination in the control period is significantly larger in cats belonging to group I No significant hemodynamic differences between the two groups was found

3 Liver metabolism

The arterial oxygen saturation in the control period averaged 90 ± 2 per cent and was not changed by the hemorrhage whereas the oxygen saturation in hepatic venous blood decreased from 51 ± 5 to 30 ± 8 per cent The increase in splanchnic oxygen extraction compensated for the decrease in EHBF, hence the splanchnic oxygen consumption which was 119 ± 13 µmol/kg/min in the control period was not significantly changed This lack of effect however, was obtained when all the experiments were pooled From Table II it appears that the splanchnic oxygen extraction increased most in cats in which hemorrhage caused an increase in ethanol elimination In these experiments (group II) the splanchnic oxygen consumption increased significantly, whereas no effect was found in the remaining experiments (group I)

The arterial blood glucose concentration which was constant in the control period (6.3 ± 1.1 mM) increased after hemorrhage and had generally reached a new level (9.1 ± 2.7 mM) within 45 min after the bleeding The splanchnic output glucose increased from 1.7 ± 0.6 to 3.5 ± 1.0 µmol/kg/min No significant differences in arterial glucose concentration or splanchnic output of glucose was found between experiments in which the ethanol elimination remained unaltered and experiments in which an increase in ethanol elimination was found The hepatic venous and arterial lactate and pyruvate concentrations were measured to estimate whether the hemorrhage was followed by hepatic or peripheral hypoxia which would change the lactate/pyruvate ratios reflecting the cytoplasmic redox level towards a more reduced state In Fig 3 the wellknown effect of ethanol on the hepatic redox level and lactate production is demonstrated The hemorrhage causes no further increase in the hepatic lactate output from the steady level in the control period The increase in arterial and hepatic venous lactate concentrations after hemorrhage is accompanied by corresponding changes in the pyruvate concentrations so that the hepatic and general redox levels were not significantly affected The response was similar in cats in which the ethanol elimination increased and in those in which no change in ethanol elimination was found

Discussion

Hepatosplanchnic hemodynamics

The hemodynamic parameters measured are in accordance with the values for cats given by Greenway and Stark (1971) except for a higher arterial fraction of the

total liver flow in the present experiments This may be explained by flow through accessory hepatic arteries in accordance with previous experiments in which the EHBF was found 10 % higher than the liver flow determined by means of electromagnetic flowmeters (Krarup 1973)

The observed decrease in gastrointestinal and total hepatic blood flow after hemorrhage agrees with the results from previous studies as reviewed by Greenway and Stark 1971 The reduced gastrointestinal flow may be due to the combined action of a decrease in perfusion pressure with intestinal (McNeill Stark and Greenway 1970) and splenic (Greenway and Stark 1969) vasoconstriction Baroreceptor mediated reflexes and sympathetic nerve stimulation caused only a short lasting effect on GIR in cats (Greenway Lawson and Mellander 1967 a) and can therefore hardly explain the intestinal vasoconstriction observed Neither an increased *catecholamine level affects the GIR to the extent found in the present experiments* (Krarup 1973) According to McNeill *et al* (1970) post hemorrhagic mesenteric vasoconstriction may be due to the release of vasopressin and angiotensin whereas splenic vasoconstriction may be the result of an increased sympathetic tone (Greenway Lawson and Stark 1968)

The observed decrease in HAR may be explained by myogenic dilatation due to the decrease in sinusoidal pressure or to accumulation of vasodilatory substances (Greenway and Stark 1971) The increase in PVR after hemorrhage which agrees with previous findings (Friedman Frank and Fine 1951 Muller and Smith 1963 Greenway *et al* 1967 b) may be due to constriction of intrahepatic portal vessels or sphincter activity in the sinusoids due to swelling of the endothelial cells (McCuskey 1966) As for the GIR increase in PVR elicited by baroreceptor mediated reflexes and sympathetic nerve stimulation was only temporary (Greenway Lawson and Mellander 1967 a) Neither infusion of catecholamines caused maintained increases in PVR (Krarup 1973) Angiotensin however may increase the PVR (Bashour *Taha and Sellers 1963) As discussed below the resistance changes in the portal* venous bed must be uniformly distributed in the present experiments in contrast to the findings in transillumination studies In some of these studies however the hemorrhage was much more severe and a more pronounced hypotension developed (Friedman *et al* 1953 Pointner *et al* 1959 Vanecko *et al* 1969)

Liver Function

The splanchnic elimination rate of ethanol reflecting the functional liver mass was not decreased by hemorrhage in the present experiments This indicates that the number of liver cells with an adequate blood supply was not changed and a gross redistribution of the liver blood flow must be excluded The decrease in plasma clearance of ICG is probably due to the decrease in hepatic plasma flow which also explains the increase in ICG extraction A decrease in the number of functioning liver cells might despite the decrease in hepatic plasma flow result in a decreased or unaltered extraction ratio of ICG

The stimulating effect of hemorrhage on ethanol elimination in 6 of the 15 cats (group II in Table II) was unexpected. The elimination rate in the control period in these was smaller than normally found in cats (Larsen 1963, Krarup 1973) but after hemorrage it returned to normal. The simultaneous increase in splanchnic oxygen consumption (Table II) may explain the effect on ethanol elimination in accordance with the view of Forsander (1963) that the elimination rate of ethanol is limited by the overall hepatic metabolism.

Liver metabolism

From the literature it appears that after moderate hemorrhages, as in the present experiments, the splanchnic oxygen extraction compensates for the decrease in hepatic blood flow so that the splanchnic oxygen consumption is not changed (Heinemann *et al.* 1953, Hamrick and Myers 1955). In contrast severe hemorrhage caused a 50 % decrease in splanchnic oxygen consumption in dogs (Frank *et al.* 1962). The increase in splanchnic oxygen consumption in 6 of the present experiments cannot be explained. Infusion of catecholamines, which increased the splanchnic oxygen consumption in man (Bearn, Billing and Sherlock 1951), was without effect in ethanol metabolizing cats (Krarup 1973). However, hemorrhage may in these experiments have released other stimulators of hepatic oxidation such as glucagon or glucocorticoids.

The rise in arterial blood glucose and splanchnic glucose output is probably a result of an increased catecholamine level after hemorrhage. This may also explain the rise in the lactate and pyruvate concentrations without changes in the lactate/pyruvate ratios in arterial and hepatic venous blood, reflecting a peripheral glycolysis. In contrast lactacidaemia due to hypoxia in the periphery or even in minor parts of the liver would be accompanied by an increase in the lactate/pyruvate ratios (Oliva 1969). Furthermore even localized hepatic hypoxia would be reflected in an increased splanchnic lactate output, in contrast to the present findings.

Conclusion

Conclusion

A hemorrhage of 15 ml/kg in cats, corresponding to about 25 % of the blood volume, caused pronounced changes in hepatosplanchnic hemodynamics with increases in gastrointestinal and portal venous resistances and a decrease in hepatic arterial resistance. Judged from the ethanol elimination and ICG extraction hemorrhage did not decrease the functional capacity of the liver and there was no indication of hepatic hypoxia after the hemorrhage. Consequently the present experiments do not support transillumination studies in which it was demonstrated that hemorrhage caused a redistribution of the hepatic blood flow, resulting in a decrease in the number of perfused sinusoids or excessive decrease in the flow through some of the sinusoids. These discrepancies may to some extent be explained by a more severe bleeding in the transillumination studies.

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References

Bashour, F A. R A. Taha and D P Sellers Effects of angiotensin on hepatic circulation in dogs *J clin Invest* 1963 *42* 916

Bearn A G B Billing and S Sherlock The effect of adrenaline and noradrenaline on hepat c blood flow and splanchnic carbohydrate metabolism in man *J Physiol* (Lond) 1951 *430—441*

Frank E D H A Frank S W Jacob and J Fine Hepatic blood flow in experimental shock *Amer J Physiol* 1962 *202* 7—11

Forsander O A Influence by alcohol on the general metabolism of the body In *Alcohol and civilization* S P Lucia, Ed McGraw Hill New York 1963 p 43—60

Friedman E W H A Frank and J Fine Portal circulation in experimental hemorrhage *Ann Surg* 1951 *134* 70—79

Friedman E. W S Milrod H A Frank and J Fine Hepatic circulation in hemorrhagic shock in the rat *Proc Soc exp Biol* (N Y) 1953 *82* 636—638

Greenway C V A E Lawson and S Mellander, The effects of the hepatic nerves in fusions of noradrenaline and occlusion of the carotid arteries on liver blood flow in the anesthetized cat *J Physiol* (Lond) 1967 a. *192* 21—41

Greenway C V A E Lawson and R D Stark The effect of hemorrhage on hepatic artery and portal vein flows in the anesthetized cat. *J Physiol* (Lond) 1967 b *193* 375—379

Greenway C V A E Lawson and R D Stark Vascular responses of the spleen to sympathetic nerve stimulation in the anesthetized cat *J Physiol* (Lond) 1968 *194* 421—433

Greenway C V and R. D Stark Vascular responses of the spleen to rapid hemorrhage in the anesthetized cat *J Physiol* (Lond) 1969 *204* 169—179

Greenway C V and R D Stark Hepatic vascular bed *Physiol Rev* 1971 *51* 23—65

Hamrick L W and J D Myers The effect of hemorrhage on hepatic blood flow and splanchnic oxygen consumption of the dog *Circulat Res* 1955 3 65—72

Heinemann H O C M Smythe and P A Marks Effects of hemorrhage on estimated hepat c blood flow and renal blood flow in dogs *Amer J Physiol* 1953 *174* 352—356

Ho M S L Responses of hepatic micro essels *Quart J Physiol* 1972 *57* 226—237

Krarup N and J A Larsen The effect of slight hypothermia on li er function as measured by the elimination rate of ethanol the hepatic uptake and excretion of Indocyanine green and bile formation *Acta physi l scand* 1972 *84* 396—407

Krarup N The effect of noradrenaline and adrenaline on hepatosplanchn c hemodynamics functional capacity of the liver and hepatic metabolism *Acta physiol scand* 1973 *87* 307—319

Larsen J A Elim nation of ethanol as a measure of the hepatic blood flo v in the cat *Acta phys ol scand* 1963 *57* 209—223

Mc Cuskey R S A dynamic and static study of hepatic arterioles and hepatic sphincte s *Amer J Anat* 1966 *119* 455—478

McNeill J R R D Stark and C V Greenway Intestinal vasoconstriction after hemo rhage roles of vasopress n and angiotensin *Amer J Phys ol* 1970 *219* 1319—1347

Muller W and L. L. Smith Hepatic arterial and portal venous circulatory changes follo v ing acute hemorrhage in the dog *Su g gynecol obstet* 1963 *117* 753—758

Oliva P B Lactic acidos s *Amer J Med* 1970 *48* 209—225

Olsen C An enzymat c fluorometric m cromethod for the d t rm nation of acetoacetate β h droxybutyrate pyruvate and lactate *Clin ch m Acta* 1971 *33* 293—300

Pointner H L. Peschl and N Stefenelli Uber Zusammenhange zwischen der Senkung des arteriellen und des portalen Blutdrucks und dem Stromungsstillstand in den Lebersinusoiden *Z g s e p Med* 1970 *152* 244—250

Seneviratne R. D Phys ological and pathological responses in the blood essels of the l e *Qua t J exp Phys ol* 1950 *35* 77—110

Siggaard Andersen O K Jorgensen and N Nerup Spectrophotometric determ nat on of o ygen saturat on in cap llary blood *Scand J clin Lab In est* 1962 *14* 98—302

Vanecko R M P B Szanto and W C Shoemaker Microcirculatory ch nges in p imate l ve during hock. *Surg Gyn Obst* 1969 *129* 995—1004

Acta Physiol scand 1973 89 278—282

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Uncompetitive Character of Inhibition by Prostaglandin E_2 of the Enhancing Effect of α-Adrenoceptor Blocking Drug on Noradrenaline Secretion in Isolated Guinea-Pig Vas Deferens

By

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Abstract

STJÄRNE L *Uncompetitive character of inhibition by prostaglandin E of the enhancing effect of α-adrenoceptor blocking drug on noradrenaline secretion in isolated guinea pig vas deferens* Acta physiol scand 1973 89 278—282

Prostaglandin E_2 has been shown to antagonize the enhancing effect of α adrenoceptor blocking drugs on the nerve stimulation induced secretion of sympathetic neurotransmitter In the present study the antagonism by PGE_2 of the effect of the α adrenoceptor blocking drug phentolamine on the secretion of noradrenaline from isolated superfused guinea pig vas deferens was found not to be competitive the two agents clearly have different targets of action on sympathetic neurotransmitter secretion In other experiments it has been shown that prejunctional α adrenoceptors may be involved in the control of the secretion of noradrenaline from sympathetic nerves by a dual mechanism one dependent and one independent of endogenous prostaglandins The present results may imply that these physiological control mechanisms are complementary and directed towards different targets in neuronal excitation secretion coupling

It is well known that α adrenoceptor blocking drugs such as phenoxybenzamine enhance the nerve stimulation induced efflux of noradrenaline (NA) from various isolated tissues (Brown and Gillespie 1957) This effect (Hedqvist 1969) as well as the enhancement of the concomitant outflow of the enzyme dopamine β hydroxylase (Johnson *et al* 1971) is prevented or depressed by low concentrations of exogenous prostaglandin E_1 and E_2 (PGE_2) The possibility has been discussed that the observed antagonism may be due to competition between α adrenoceptor blocking agents and PGE for prejunctional sites regulating the secretion of neurotransmitter (Johnson *et al* 1971)

The present experiments were carried out to study the nature of the antagonism between the reversible competitive α adrenoceptor blocking drug phentolamine (PA) and PGE_2 on the secretion of NA from isolated superfused field stimulated guinea

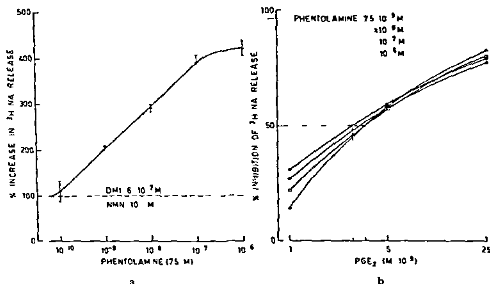


Fig 1 A Enhancement by phentolamine (PA) of secretion of ^{3}H NA on nerve stimulation at 5 Hz in isolated superfused field stimulated guinea pig vas deferens in relation to level obtained by addition of desipramine (DMI) and normetanephrine (NMN) to block reuptake of NA Means and range of 2 observations at each concentration of PA

Fig 1 B Relative inhibitory effect of PGE on NA secretion at different concentrations of PA Each point represents the average of 2—7 observations

pig vas deferens The results clearly show that the antagonism between PA and PGE₂ on this function is not competitive

Material and Methods

The experiments were carried out in 12 guinea pigs weighing 250—300 g as described elsewhere (Stjarne 1973 a) NA secretion was monitored by determination of the nerve stimulation induced rise in efflux of total ^{3}H after preincubation of the tissue with 10 μCi/ml of ^{3}H l NA (New England Nuclear Corp specific activity 6 5 C /mmol) Desipramine (DMI) 6×10^{-7} M and normetanephrine (NMN) 10^{-5} M were present in the superfusion medium Tyrode solution to block reuptake of NA (*cf* I ersen 1967) NA secretion was induced by field stimulation with biphasic pulses of near maximal strength and a duration of 1 5 ms Trains of 300 pulses ere applied at 5 Hz with about 10 min intervals Muscular contraction was recorded by means of an isotonic transducer (Harvard) loaded to balance resting tone recordings ere made on a Grass polygraph Total ^{3}H activity in 1 ml aliquots of the uperfusate was determined by counting for 10 min in 10 ml of Instagel (Packard) in an APAC SL 40 Intertechnique Liquid Scintillation Spectrometer Since ^{3}H NA accounted for more than 90 % of the total radioactivity in the tissue since the nerve stimulation induced rise in efflux of ^{3}H could by completely abolished by addition of tetrodotoxin 5×10 g/ml and since reuptake of released NA was blocked by DMI and NMN it is assumed that the rise in efflux of total ^{3}H reflects total nerve stimulation induced secretion of ^{3}H NA

The following drugs were used Desipramine (Pertofran® Geigy) normetanephrine (NMN Sigma) phentolamine sulphonate (CIBA) and prostaglandin E₂ from D P ke Upjohn Co)

Results

Even in the presence of DMI and NMN to block reuptake of NA released by nerve stimulation PA further enhanced the nerve stimulation induced efflux of total ^{3}H

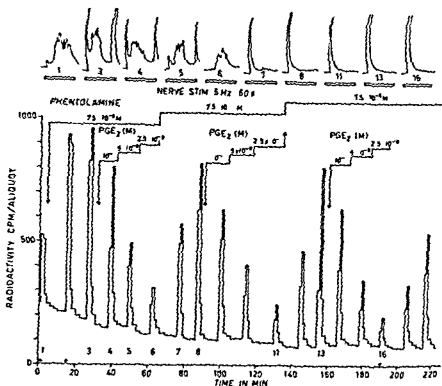


Fig 2 Typical experiment showing the muscular contraction in response to nerve stimulation (upper panel) and the nerve stimulation induced rise in efflux of total ^3H in superfusate (lower panel) For details see Text

in a dose dependent manner indicating that PA caused enhanced secretion of ^3H NA (Fig 1 A)

As seen in Fig 2 which shows a typical experiment PA 7.5×10^{-8} M strongly enhanced the nerve stimulation induced secretion of ^3H NA this effect was antagonized in a dose dependent manner by low concentrations of PGE After raising PA to 7.5×10^{-7} M and waiting for about 20 min when the depressing effect of the previous infusion of PGE_2 was fading off a second infusion of PGE_2 was again found to depress the nerve stimulation induced secretion of ^3H NA to about the same extent as at PA 7.5×10^{-8} M Finally the sequence was repeated at the supramaximal PA concentration (cf Fig 1 A) of 7.5×10^{-6} M the relative inhibitory effect of PGE was about the same as at the two lower concentrations of PA

The initial rapid twitch like contractile response to nerve stimulation was not blocked but its latency was shortened and its amplitude was enhanced to a progressively increasing extent by increasing doses of PA (Fig 2 upper panel note particularly stimulations 1 3 8 and 13) At PA 7.5×10^{-8} M the delayed contractile response was not markedly reduced either (Fig 2 stimulation 3) while it became completely abolished at higher concentrations of PA (Fig 2 stimulations 8 13)

At the lowest concentration of PA, 7.5×10^{-8} M PGE markedly delayed and depressed or abolished the initial twitch while the delayed contraction was less affected (Fig 2 stimulations 3—6) At the highest concentration of PA 7.5×10^{-6} M PGE delayed the twitch but rather appeared to enhance its amplitude and duration (Fig 2 stimulation 13 16)

The relative inhibitory effect of PGE on nerve stimulation induced secretion of ^{3}H NA was thus found to be independent of the concentration of PA (Fig 1 B) the ID$_{50}$ for PGE on this function was about 3.3×10^{-9} M at concentrations of PA varied from 7.5×10^{-9} M to 7.5×10^{-6} M

Discussion

The present results confirm and extend previous observations of antagonism between PGE and α adrenoceptor blocking drugs on nerve stimulation induced secretion of NA in perfused cat spleen (Hedqvist 1969) and in guinea pig vas deferens (Johnson *et al* 1971 Stjarne 1973 a) The results definitely show that the antagonism between PA and exogenous PGE is not competitive the two agents clearly act on different targets in neuronal excitation secretion coupling

The results may also have implications concerning the physiological control of sympathetic neurotransmitter secretion As shown in previous work on guinea pig vas deferens it appears that 2 separate mechanisms triggered by probably prejunctional α adrenoceptors exert negative feed back control on NA secretion one seems to be dependent and one independent of endogenous PGE (Stjärne 1973 b) The present observation that low concentrations of PA which do not depress but rather enhance nerve stimulation induced muscular contraction still strongly elevate the secretion of NA suggests that PGE released as a consequence of stimulation of post junctional α adrenoceptors is probably not in the position to strongly affect NA secretion from the nerves This fact plus the finding that release of endogenous PGE to restrict NA secretion does not appear to require muscular contraction (Stjärne 1972) rather strongly suggest that the specific perhaps small fraction of total endogenous PGE which seems to be involved in the physiological control of NA secretion is derived from non muscular sources and quite possibly from the nerves themselves

Prejunctional α adrenoceptors appear to play a pivotal role in the physiological control of NA secretion from sympathetic nerves The present results suggest that the two different mechanisms involved one dependent and one independent of endogenous PGE (Stjarne 1973 b) are complementary and directed towards different targets in the sequence of events starting with the invasion of sympathetic nerve terminal by propagated action potentials and ultimately to an apparently regulated extent leading to successful activation of local excitation secretion coupling mechanisms in individual secretory varicosities

This work was supported by a grant from the Swedish Medical Research Council (project B73-04X 3027 04) I want to thank my wife Mrs Eio Stjarne for excellent technical assistance

References

BROWN G L and J S GILLESPIE The output of sympathetic transmitter from the spleen of the cat *J Physiol* (Lond) 1957 *138* 81—102
HEDQVIST P Antagonism between prostaglandin E_2 and phenoxybenzamine on noradrenaline release fron the cat spleen *Acta physiol scand* 1969 *76* 383—384
IVERSEN L L *The uptake and storage of noradrenaline in sympathetic nerves* London Cambridge University Press 1967 p 147
JOHNSON D J N B THOA R WEINSHILBOUM J AXELROD and I J KOPIN Enhanced release of dopamine β hydroxylase from sympathetic nerves by calcium and phenoxybenzamine and its reversal by prostaglandins *Proc Nat Acad Sci* (Wash) 1971 68 2227—2230
STJARNE L Prostglandin E restricting noradrenaline secretion neural in origin? *Acta physiol scand* 1972 *86* 574—576
STJARNE L Lack of correlation between profiles of transmitter efflux and of muscular contraction in response to nerve stimulation in isolated guinea pig vas deferens *Acta physiol scand* 1973 a *88* 137—144
STJARNE L Dual alpha adrenoceptor mediated control of secretion of sympathetic neurotransmitter one mechanism dependet and one independent of prostaglandin E *Prostaglandins* 1973 b *3* 111—116

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Sympathectomy and Pharmacological Blockade in Trained Rats

By

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Several investigations have been made both in humans and in animal to analyze the mechanisms causing the resting bradycardia and the relative bradycardia during exercise evoked by physical training Some investigators have used trained rats for this purpose although only with measurements of heart rate at rest (Östman and Sjöstrand 1971 Tipton 1965)

In this study measurements of the heart rate of rats were made at different levels of exercise before and after training The purpose was to examine the effectiveness of running on a treadmill in producing relative bradycardia during exercise Further investigations about the bradycardia of training were also made by performing work tests after pharmacological blockade of the different portion of the autonomic nervous system

3 small silver electrodes were implanted subcutaneously on the back of 0 rats female outbred Sprague Dawley ?00 g These electrodes could easil be connected to cables for ECG recordings at rest and during exercise After healing of the operation wounds the rats were familiarized with running on a treadmill at low speed 15 min a day for a week.

Exercise heart rate measurements at 3 standard work loads were performed with all the rats before and after the training period (see Fig 1) 10 of the rats randoml selected were trained on a treadmill for about 1/2 h a day 5 days a week at continuously increasing speeds from 15 to 30 m/min for 6 weeks while the other 10 served as control

1 to ? days after the post training test 5 rats from each group were treated with 6-hydroxydopamine 6-OH DA 50 mg/kg i v This drug gives an acute sympathomimetic effect and a longlasting degeneration of the sympato-adrenergic nerves (Malfors and Thoenen 19 1) The influence on the heart rate was measured at rest for ? h immediately following the injection On the following da the same work test as used previousl was performed

With the rest of the rats, 5 trained and 5 controls parasympathetic blockade was accomplished by injection of atropine sulphate 2 mg/kg c The next da the drug H 93/26 a cardio-selective β blocker without intrinsic activity Ablad (a sson and Ek 19 3 was administered (20 mg/kg i.p) to the same rats Work test were performed 15—30 min after the atropine injection and about 30 min afte the H 93 [illegible] injection respectively

After the experiments the rats were killed and the hearts of [illegible] were weighed

The differences between the mean value of the trained and untrained groups were calculated and tested according to Student s t test Level of significance $P < 0.05$

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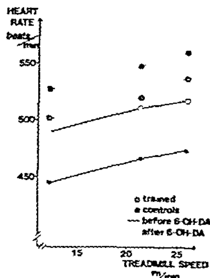


Fig 1 Exercise heart rates of 5 rats before and one day after the injection of 6-OH DA Testing procedure running for 10 min at 12 m/min and subsequent running for 5 min at 21 and 25 5 m/min with heart rate recordings at the last 3 min of each period

The heart rates of the rats increased proportionally to the increasing work intensity (Fig 1)

Before training the heart rates of the 2 groups were identical at the treadmill test After the experimental period the heart rate reaction of the control group remained unchanged while the mean heart rate of the trained group had decreased by 40—beats/min at all test speeds (Fig 1)

The heart weights of the trained rats were slightly higher than the heart weights of controls (Trained mean heart weight 0.93 g SD 0.07 g Controls mean heart weight 0.87 g SD 0.07 g) The difference was not statistically significant

One day after the 6-OH DA injection the heart rates at the exercise test had increased significantly because of denervation supersensitivity ($p < 0.05$) and the difference between the 2 groups (5 rats in each) had decreased although not significantly (Fig 1) The heart rates of the atropinized animals increased about 40 beats/min at all work loads and the increase of the 2 groups was equal After treatment with H 93/26 the heart rates during exercise decreased with more than 100 beats/min but the difference between the trained and the sedentary group was completely unchanged

The acute effect of 6-OH DA was a decrease in resting heart rate immediately after the injection The decrease was much more pronounced in the control group about 140 beats min than in the trained one about 50 beats min ($p < 0.05$) After 2 h the difference between the groups had disappeared

This pilot study shows that a 6 week training period was enough to produce a considerable decrease in exercise heart rate of rats The mean heart weight difference between trained and untrained rats was of approximately the same size as other authors have shown (Ostman and Sjostrand 1971) although our material was too small to give statistically significant results

Some factors that theoretically could be involved in producing bradycardia in trained individuals are alone or in combination

1) Changes in activity of the autonomic nervous system
2) Changes in the level of circulating catecholamines from the adrenals or
3) Changes in the heart itself *e g* cardiac hypertrophy or decreased sensitivity to transmittor substances

After elimination of the adrenergic nerves with 6 OH DA there still remained a difference in working heart rate between the trained and the sedentary group although the difference was less pronounced than before the blockade This indicates that changes in activity of the adrenergic nerves had probably occurred although it was not the sole cause of the bradycardia

The parasympathetic nervous system did not seem to be the only factor causing bradycardia because after atropine treatment there still existed a difference in heart rate response to exercise between the trained and untrained groups

After elimination of the influence of the circulating catecholamines on the heart rate by beta adrenergic blockade there still was some mechanism maintaining the difference Therefore neither the adrenergic nervous system nor the parasympathetic nervous system nor the circulating catecholamines seemed to be alone responsible for the bradycardia evoked by physical training Similar results have recently been obtained in training experiments on man (Ekblom Kilbom and Soltysiak 1973)

The less pronounced acute effect of 6 OH DA on the trained group at rest indicates that a decreased sensitivity to catecholamines in these animals could be a part of the adaptation mechanism This and other factors and combinations between them are presently subject to further investigations

References

ABLAD B E CARLSSON and L. EK, Pharmacological Studies of Two New Cardio-selective Adrenergic Beta Receptor Antagonists *Life Sci* 1973 *12* 107—119

EKBLOM B A KILBOM and J SOLTYSIAK Physical training bradycardia and autonomic nervous system. *Scand J clin Lab Invest* 1973 In press

MALMFORS T and H THOENEN (ed) *6 hydroxydopamine and Catecholamine Neurons* North Holland Publ Co 1971

ÖSTMAN I and N O SJOSTRAND Effect of Prolonged Physical Training on the Catecholamine Levels of Heart and the Adrenals of the Rat *Acta physiol scand* 1971 *82* 202—208

TIPTON C M Training and Bradycardia in Rats *Amer J Physiol* 1965 *209* 1089—1094

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Evidence for Adrenaline Neurons in the Rat Brain

By

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Adrenaline has been found in the rat brain in biochemical investigations (Vogt 1954 Gunne 1962) Furthermore Ciaranello et al (1969) and Pohorecky et al (1969) were able to demonstrate enzymatic adrenaline synthesis in the brain of various mammals

In spite of the fact that adrenaline principally can be visualized with the formaldehyde fluorescence (Falck Hillarp) technique no histochemical evidence has so far been presented for adrenaline neurons in the mammalian brain probably due to difficulties in differentiating between various catecholamines with this method

The introduction of immunohistochemistry into neurotransmitter research (Geffen et al 1969) has opened up new possibilities to identify and localize monoamine neurons In the present study we have used antibodies to phenylethanolamine N methyltransferase (PNMT) the enzyme converting noradrenaline to adrenaline in an attempt to trace the possible cellular localization of this enzyme in the rat brain with the indirect immunofluorescence technique

A specific staining of certain neurons was obtained after incubation with antiserum to PNMT followed by incubation with fluorescein isothiocyanate conjugated antibodies The cell bodies were localized in two groups in the medulla oblongata The first one (Group C1) was localized in the ventrolateral reticular formation of the rostral medulla oblongata laterally of the olivary complex and caudal to the nucleus n facialis (Fig 1) This distribution is very similar to the catecholamine cell bodies in the rostral part of group A1 The second cell group (Group C2) was also found at the rostral level of the medulla oblongata mainly surrounding the medial part of the ventral surface of the fourth ventricle

Nerve terminals were found in various nuclei of the brain stem often in periventricular area A dense network of PNMT positive nerve terminals were found in the nucleus motorius dorsalis n vagi (Fig 2) nucleus tr solitarii nucleus paraventricularis magnocellularis nucleus paraventricularis rotundocellularis thalami and also in the lateral sympathetic nucleus of the spinal cord A moderate density was ob-

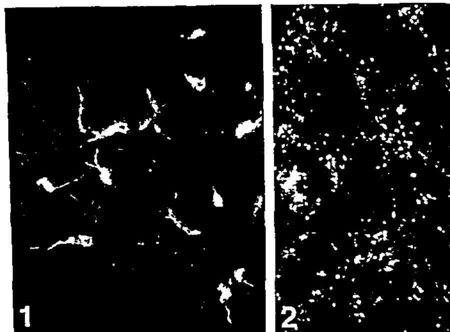


Fig 1 Immunofluorescence micrograph of section of the medulla oblongata incubated with antiserum to PNMT and fluorescein isothiocyanate conjugated antibodies (indirect technique) Several fluorescent cell bodies with processes a e seen in the entrolateral reticular formation group C1) Magnification 160 ×

Fig 2 Immunofluorescence micrograph of the nucleus motorius dorsali n vagi. Same technique as in Fig 1 A dense plexus of PNMT positive nerve terminals is seen in the neurop l between the neurons of thi nucleus Magnification 320 ×

erved e g in the perifornical area nucleus dorsomedialis hypothalami periventricular grey matter in the mesencephalon diencephalon and spinal cord

The present results demon trate in the rat brain the existence of a well defined neuron system con aining an enzyme which reacts immunologically with antibodies to bovine adrenal PNMT The cell bodies are locali ed in the reticular formation of the medulla oblongata and give ri e to both ascending and descending axons with terminal plexus in the pons me encephalon diencephalon and spinal cord It is suggested that these neurons contain PNMT and that they are capable of synthesizing adrenaline which may be utilized as a neurotransmitter

The present stud was supported by the Swed h Medical Rese h Council (B77 14X 887 04C B/7 04X /15-8A) by Magnus Bergvalls Stiftelse and by USPHS grant MH-02717 and by NSF grant GB-8465

References

CIARANELLO R D, R E BARCHAS, G S BYERS, D W STEMMLE and J D BARCHAS Enzymatic synthesis of adrenaline in mammalian brain *Nature* (Lond) 1969 *221* 368—369

GEFFEN L B, D G LIVETT and R A RUSH Immunohistochemical localization of protein components of catecholamine storage vesicles *J Physiol* (Lond) 1969 *204* 593—605

GUNNE L M, Relative adrenaline content in brain tissue *Acta physiol scand* 1962 *56* 324—333

POHORECKY L A, M ZIGMOND, H KARTEN and R. J WURTMAN Enzymatic conversion of norepinephrine to epinephrine by the brain *J Pharmacol exp Ther* 1969 *165* 190—195

VOGT M The concentration of sympathin in different parts of the central nervous system and normal conditions and after the administration of drugs *J Physiol* (Lond) 1954 *123* 451—481

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Intrarenal Distribution of Glomerular Filtration in Conscious Rats during Isotonic Saline Infusion

By

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Abstract

CLAUSEN, G. and I. TYSSEBOTN. *Intrarenal distribution of glomerular filtration in conscious rats during isotonic saline infusion.* Acta physiol. scand. 1973. *89.* 289—295.

Relative individual glomerular filtration rate (gfr) of juxtamedullar (JM) and superficial (S) nephrons was determined in conscious rats by the ^{14}C ferrocyanide technique. Isotonic saline infused at 50 μl/min×100 g b.w. for about 60 min reduced fractional Na reabsorption by 11 % with no significant change in inulin clearance. The S gfr averaged 90 % of JM gfr both in control and saline loaded animals, but with less scatter in the latter group. Thus the natriuresis produced by acute i.v. isotonic saline loading in conscious rats is not caused by redistribution of filtrate.

Extracellular volume expansion
Individual nephron filtrate
Saline loading
Sodium excretion
^{14}C Sodium ferrocyanide

The reduction of the renal fractional sodium reabsorption occurring in rats acutely expanded by i.v. saline infusion may be associated with, and possibly caused by, redistribution of filtrate from juxtamedullary (JM) to superficial (S) nephrons. Such redistribution has been demonstrated by micropuncture technique (Andreucci *et al.* 1971, Davidman *et al.* 1971, Jamison and Lacy 1971) and by the ferrocyanide technique (Barnes and Davis 1970, Coelho *et al.* 1970).

Since these experiments were performed on anesthetized animals, usually with the experimental kidney exposed, the possibility remained that the observed redistribution of filtrate during saline loading might not be representative for intact unanesthetized animals.

In the present study, therefore, the effect of isotonic saline infusion on the relative filtration rates of superficial and juxtamedullary nephrons was determined in non-anesthetized rats without kidney exposure by means of the ferrocyanide technique (Hanssen 1958, 1963).

Methods

The experiments were made on albino Wistar rats weighing 250—400 g with free access to standard rat food and water. Catheters were inserted in a femoral vein (PE 50) and artery (PE 10) and in the bladder (PE 50) through a small suprapubic incision. The preparations took about 20 min and were made during ether anesthesia. The rats were restrained in a cage made of plexiglass tube of 60 mm inner diameter, split transversely in two sections just anterior to the kidneys. The hind limbs protruding trough a wide hole in the cage were secured by tape in flexed position 8 cm apart. This arrangement permitted guillotining the rat at the level of the diaphragm to stop renal blood flow and filtration at desired time after ferrocyanide injection (cf. below). To avoid disturbing the restrained rats when sampling blood and urine the sides and rear of the cage were covered. Except for occasional incidents of struggling in some animals early in the 45 min equilibration period the rats remained quiet in the cage.

Blood pressure was recorded with a Statham pressure transducer and a Sanborn recorder. Blood samples of 0.5 ml were collected from the arterial catheter. A priming dose of 100 µl/100 g b.w. and sustained infusion of 5 µl/min × 100 g b.w. of a solution containing 6 parts 10 % inulin, 5 parts serum and 4 parts 5.5 % glucose were given through the venous catheter. Serum was added in volumes calculated to compensate for blood sampling during the experiment. The rats recovered from ether anesthesia within 10 min after surgery. The sampling periods began after an equilibration period of about 45 min. In the control group three 60 min clearance periods with mid period blood sampling preceeded the sodium ferrocyanide injection. In the second group natriuresis was produced after two 60 min control period by an infusion of 0.9 % sodium chloride at a rate of 250 µl/min × 100 g b.w. for about 60 min. During saline loading 10—20 min clearance periods were used.

The relative individual glomerular filtration rate (gfr) was determined by the method of Hanssen (1958, 1963). [illegible] 00 µCi ^{14}C labelled sodium ferrocyanide in a 20 % solution of non-labelled sodium ferrocyanide was injected through the venous catheter in the course of 2 s. 8—12 s after the injection the rat was guillotined. The kidneys thus exposed were rapidly excised, cut into 2—3 mm thick radial slices and frozen in isopenthane cooled in liquid nitrogen or in alcohol-solid CO_2 mixture. The slices were left in alcoholic ferric chloride (50—60 g hydrated ferric chloride in 95 ml absolute alcohol and 5 ml concentrated hydrochloric acid) for 16 h, washed and macerated in 20 % hydrochloric acid for 6 h at about 45 C. They were then washed three times and stored in 0.2 % ferric chloride 1 % acetic acid solution at 4 C. Dissection of the nephrons in the storage fluid was made within a few days. In order to minimize loss in selecting tubules the dissection was performed in [illegible] light making the Prussian blue precipitate invisible.

During dissection of nephrons it soon became clear that an exact localization of the glomerulus of each nephron dissected from a given cortex could not be obtained. Single nephrons could not be dissected free from the intact macerated kidney slice. Instead we first removed and loosened group including both types of nephrons and secondly disentangled the individual nephron. This procedure disrupted the [illegible] configuration. However it was observed that nephrons with deep and superficially located glomeruli had characteristic tubular pattern that could be easily recognized. The superficial nephron had tightly folded proximal convolutions and a well defined straight segment constituting about half the length of the proximal tubule whereas juxtamedullary nephrons were found to have more loosely folded convolutions and to lack a well defined straight segment. Intermediates were discarded.

In order to approximate the [illegible] of [illegible] other [illegible] dissected tubule segments of distal tubules corresponding roughly to proximal tubular length were included in each series. Each series [illegible] superficial juxtamedullary and distal tubules, about 10 of each were usually dissected in each rat. Three [illegible] glomeruli each were also included. In order to minimize the effect of possible uneven regional distribution of the ferrocyanide bolus in the kidney [illegible] were taken from three separate sectors of one radial kidney slice in most rats.

The dissected proximal tubules including glomeruli were broken at the beginning of the thin segment. They contained all the filtered ferrocyanide since the [illegible] precipitate never reached the last 30—50 % of the proximal tubules. The tubules were transferred on needle tip to vials containing 0.2 ml tissue [illegible] Solvent). 10 ml scintillation fluid (4 g PPO and 50 mg POPOP per litre toluene) was added and the [illegible] was determined in a Beckman liquid scintillation [illegible] [illegible] was counted for 20 min.

Inulin was determined photometrically with anthrone [illegible] sodium [illegible] absorption photometry (Perkin Elmer Mod. 303) [illegible] (Advanced Instruments Osmometer 3L).

TABLE IA Renal functions in a natriuretic rat (No 4 in Table II)

Min	AP mm Hg	P_{Na} μeq/ml	V μl/min × 100 g	U_{osm} mosm/l	U_{Na} μeq/ml	$U_{Na} \times V$ μeq/min × 100 g	C_I [ml/min] × 100 g	$C_I \times P_{Na}$ μeq/min × 100 g	Na reabs % of filtered
0–55	127	147	7	1045	66	0.46	1.25	184	99.8
55–115	127	147	8	1000	68	0.55	1.10	162	99.7
115	Start i.v. saline infusion 250 μl/min × 100 g b.w.								
115–133	125	149	18	861	82	1.43	0.94	140	98.8
133–148	122	148	84	374	73	6.13	1.05	155	96.0
148–160	124	148	195	258	56	10.9	1.12	166	93.4
160–171	125	147	174	335	104	18.0	1.16	170	89.4
171–183	122	148	176	330	95	16.7	1.26	185	91.0

Effective volume expansion was 7 % of b.w.

TABLE IB dl S gfr determined in one series of tubules dissected from a natriuretic rat (No 4 in Table II)

	Total β activity cpm*			gfr in % of mean JM D cpm	
	JM	S D	JM	JM	S-dl
Mean	311	290 82		100	89
S E				4.9	7.2

* Reagent blank 70 cpm

Results

Table I shows the results from a typical experiment. The effect of saline loading on urine flow, osmolarity and sodium concentration was usually apparent during the first and fully developed during the third or fourth clearance period following saline infusion (Table IA).

The results from hydropenic and natriuretic rats are shown in Table II. The mean arterial blood pressure was 126 (120—135) mm Hg, individual variations being within ±5 mm Hg in both groups. Neither saline infusion nor the rapid sodium ferrocyanide injection affected blood pressure. The average inulin clearance (C_{In}) was 0.87 ml/min × 100 g b.w. in the control group and 1.14 ml/min × 100 g b.w. during the control period in the saline loaded group. However, C_{In} did not increase during the saline infusion, the average value being 1.15 ml/min × 100 g b.w. during the last 10—20 min collection period. The urine flow increased tenfold or more to an average of 160 μl/min × 100 g b.w. and the sodium excretion reached 17.6 (12.0—25.2) μeq/min × 100 g b.w. Fractional sodium reabsorption was on average reduced to 89 % compared to practically complete reabsorption prior to saline infusion. Effective volume expansion determined as infused volume minus urine production and blood samples was 5.5 (2—10) % of b.w. at the time of sodium ferrocyanide injection.

TABLE II Renal functions in conscious rats during hydropenia and natriuresis produced by i.v. infusion of isotonic saline at 250 µl/min × 100 g b.w. for about 60 min

Weight g	U_{osm} mosm/l	V µl/min × 100 g	C_I ml/min × 100 g	Na reabs % of filtered	Volume exp % of b.w.	S gfr ± S.D. % of JM gfr	n S/JM nephrons
*Hydropenia**							
334	283	22	1.05	99.9	—	91 ± 19	19/19
254	1030	7	0.70	99.9	—	81 ± 40	18/18
272	862	7	0.70	99.8	—	87 ± 16	18/18
361	701	8	0.87	99.9	—	76 ± 3	5/5
279	644	16	0.94	99.8	—	89 ± 27	18/18
243	940	11	0.94	99.9	—	100 ± 26	18/18
Mean							
291	743	12	0.87	99.9	—	90 ± 28	96/96
*Saline infusion***							
417	311	149	0.80	87.1	10	97 ± 11	10/10
370	340	184	1.42	94.1	[illegible]	90 ± 19	15/15
354	336	183	1.05	81.2	3	95 ± 10	10/10
286	330	176	1.26	91.0	[illegible]	93 ± 21	18/17
283	330	108	1.14	90.8	5	73 ± 18	21/21
Mean							
342	323	160	1.15	88.8	5.5	90 ± 19	80/73

* Mean of 2—3 clearance periods
** Values from the last 10—20 min clearance period

Proximal tubular radioactivity was about 350 counts per minute. The average radioactivity of single glomeruli and of distal tubular segments ("tubular blank") was 3—7 % and 2—31 % of JM radioactivity respectively.

The mean radioactivity of the JM proximal tubules after having subtracted tubular blank was arbitrarily set to 100 % gfr in each series of dissected nephrons in each rat. The mean S gfr averaged 90 % of JM gfr both in the control group and in the saline infused group. The S gfr was significantly different from JM gfr in both groups ($P < 0.01$ in the controls, $P < 0.005$ in the saline loaded rats) but not in individual rats ($P > 0.1$). The standard error of the mean (S.E.) was 5 gfr 2.9 % and 1.9 % JM gfr 3.6 % and 1.6 % in controls and saline loaded rats respectively, i.e. significantly lower in the latter group. The S.E. values imply that the smallest statistically significant JM gfr—S gfr difference which could have been detected would be 6.5 % in the natriuretic and 8 % in the hydropenic rats ($P = 0.05$).

Discussion

The use of Hanssen's ferrocyanide method for estimating relative individual nephron filtration rate requires that

1. Ferrocyanide arrives in all glomeruli simultaneously and in equal concentration and that the infusion does not interfere with renal blood flow or filtration rate. The intravenous injection should ascertain a complete mixing of ferrocyanide in the blood

reaching the kidney and the fact that the injection caused no change in arterial pressure also suggests minimal influence on renal blood flow and filtration rate

2 Filtered ferrocyanide must remain quantitatively within the tubules after it is filtered both *in vivo* during precipitation as Prussian blue and during maceration and dissection It has been shown that the clearance of ferrocyanide equals that of inulin in rats (Baines *et al* 1969) and that intratubular ferrocyanide due to incomplete precipitation or to dissolution of precipitate is less than 2—3 % *in vitro* (Hanssen 1958) Thus it seems well established that intratubular β activity is directly proportional to gfr

3 Intratubular ferrocyanide can be estimated without contamination with non filtered ferrocyanide Since only a fraction of the ferrocyanide passing through the kidney *is filtered in the glomeruli some of the injected dose will obviously be present* in the glomerular capillaries peritubular capillaries and interstitial fluid 8—12 s after injection indicating 2 sources of error It is not possible to decide how much of the glomerular radioactivity is located in glomerular capillaries or in Bowman s capsule *i e* to what extent glomerular radioactivity is due to filtered ferrocyanide The average radioactivity of single glomeruli was 3—7 % of the average radioactivity in JM proximal tubules and should therefore result in a few per cent over estimate of filtered ferrocyanide but should hardly affect the S to JM gfr ratio The second source of error is radioactivity of extratubular origin adhering to the proximal tubules That a similar amount of unfiltered ferrocyanide adheres to proximal tubules and comparable lengths of distal tubules (tubular blank) is indicated by the studies of Coelho *et al* (1971) The present tubular blank therefore seems to constitute the best available correction for unfiltered material The marked variation of tubular blank 2—31 % of JM radioactivity presumably depends on the shape of the ferro cyanide bolus reaching the kidney in relation to the time of stopping renal blood flow It cannot be excluded that the activity adhering to proximal tubules may differ in the two types of nephrons and thereby to some extent influence the estimation of gfr but it is very unlikely that this should mask possible changes in the JM to S gfr ratio during saline infusion Based on these considerations it seems safe to conclude that the method should detect redistribution of filtrate causing alterations of S gfr greater than 6 5—8 % (*cf* Results)

The present experiments in conscious rats demonstrated that volume expansion by i v saline infusion caused a marked natriuresis with no change in either total GFR or in single glomerular filtration rate These findings are contrary to several previous results obtained in anesthetized rats Most authors report that i v isotonic saline *loading increases total GFR whereas the corresponding results on gfr are conflict* ing Baines and Davis (1970) and Coelho *et al* (1970) using the ferrocyanide method found that S gfr increased relative to JM gfr by 11 % and 26 % respectively in saline loaded anesthetized rat Like Baines and Davis (1970) we observed a more uniform nephron function during natriuresis not in terms of increased S gfr relative to JM gfr but as a significant fall in the standard deviation of gfr in both groups of nephrons Using micropuncture technique Jamison and Lacy (1971) at

Davidman *et al* (1971) found a larger increase in S gfr than in total GFR in is saline loaded anesthetized rats, whereas Bartoli and Early (1971) Andreucci *et al* (1971) and Daugharty *et al* (1972) obtained proportional increase in S gfr and total GFR in similar experiments

The S gfr in the hydropenic rats (Baines and Davis 1970 Coelho *et al* 1970) was 67 % and 72 % of JM gfr respectively compared to 90 % in the present rats The total GFR during hydropenia in the experiments of Baines and Davis (1970) was markedly lower than in the present study This also applies to those of the other investigations referred to above where data on GFR are available One might therefore suspect that anesthesia surgery or handling of the experimental kidney reduces total GFR, but reduces S gfr more than total GFR thus giving a low S gfr in control rats Under these conditions saline loading might tend to normalize both these parametres resulting in an increased S gfr

Andreucci *et al* (1971) who found a proportional rise in total GFR and S gfr with a standard expansion procedure (mild volume expansion) also reported that "massive volume expansion produced redistribution of filtrate to S nephrons indicating that filtrate redistribution may depend on the degree of effective volume expansion Most investigators have infused 10 % of b w or more at a rate comparable to that used in the present study but the urine flow where data are available has been considerably smaller and the effective volume expansion therefore larger than in the present conscious rats (average 5 5 % of b w)

During natriuresis the fractional sodium excretion was 11 % compared to 2—5 % in the previous investigations including the massive volume expansion group of Andreucci *et al* (1971) The corresponding rate of sodium excretion was 17 6 μeq/min × 100 g b w compared to 1 9—10 3 μeq/min / 100 g b w in anesthetized rats

This comparison of urine flow and sodium excretion obtained in the various studies demonstrates that the homeostatic effect of the natriuresis which obviously must be to counteract sodium retention and expansion of extracellular volume was more pronounced in the present conscious animals In spite thereof we were unable to demonstrate any change in the relative filtration rates of superficial and juxtamedullary nephrons and must therefore conclude that natriuresis produced by intravenous isotonic saline infusion in conscious rats is not caused by redistribution of glomerular filtrate

References

ANDREUCCI V E J HERRERA ACOSTA F C RECTOR JR and D W SELDIN Effect of glomerular filtration pressure and single nephron filtration rate during hydropenia, elevated arterial pressure and acute volume expansion with isotonic saline J clin Invest 1971 50 2230—2234

BAINES A D and C DAVIS Juxtacortical shifts in glomerular tubular function influence of volume expansion In Regulation of body fluid volumes by the kidney 1970 40 55 Ed ited by Cort, J H Karger Basel

Bartoli E and L E Earley The relative contribution of reabsorptive rate and redistributed nephron filtration rate to changes in proximal tubular fractional reabsorption during acute saline infusion and aortic constriction in the rat *J clin Invest* 1971 *50* 2191—2203

Coelho J B S R Stella, K C H Chien and S E Bradley Glomerular filtration rate (GFR) in superficial (S) and juxtamedullary (JM) nephrons during salt loading and hemorrhagic hypotension *Clin Res* 1970 *18* 496

Coelho J B K C H Chien S R Stella and S E Bradley Relationship of extraluminal tubular deposition of ferrocyanide to peritubular perfusion rate in cortical and medullary nephron segments of the rat kidney *Circulat Res* 1971 *29* 21—28

Daugharty T M I F Ueki D P Nicholas and B M Brenner Comparative renal effects of isoncotic and colloid free volume expansion in the rat *Amer J Physiol* 1972 *222* 225—235

Davidman M R C Lalone E A Alexander and N G Levinsky Proximal function in volume loading in the rat *Clin Res* 1971 *19* 528

Führ J J Kaczmarczyk and C D Krüttgen Eine einfache colorimetrische Metode zur Inulinbestimmung für Nieren Clearance Untersuchungen bei Stoffwechselgesunden und Diabetikern *Klin Wschr* 1955 *33* 729—730

Hanssen O E A histochemical method for evaluation of excreted sodium ferrocyanide in isolated tubules of the mouse kidney *Acta path microbiol scand* 1958 *44* 363—371

Hanssen O E Method for comparison of glomerular filtration in individual rat nephrons *Proc 2nd Int Nephrol Congr* 1963 pp 527—529

Jamison R L and F B Lacy Effect of saline infusion on superficial and juxtamedullary nephrons in the rat *Amer J Physiol* 1971 *221* 690—697

Acta physiol. scand. 1973. 89. 296—305

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Release of ³H-Noradrenaline from Incubated and Superfused Rabbit Pulmonary Artery

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Abstract

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The quantitative release of (—)-³H-noradrenaline (³H-NA) from rabbit pulmonary artery was studied and a comparison made between a superfused strip and an incubated ring preparation. Two rings, but only one strip, could be derived from a single pulmonary artery. The postganglionic adrenergic neurones were loaded with ³H-NA and the release of tritium caused by either transmural stimulation or drugs was followed by fractional collection. The concomitant tension response was also monitored. The amount of tritium released per ml fraction elicited by transmural stimulation, nicotine and tyramine were 1.5, 2.5 and 5 times higher, respectively, using the incubated ring. Total tritium release induced by transmural stimulation or nicotine was equal using the two methods. In contrast, the release caused by tyramine was [illegible] times higher with the incubated ring preparation. Retention of ³H-tyramine by the superfused strip was slightly higher compared to the incubated ring. The sensitivity of the smooth muscle to either exogenously added or endogenously released noradrenaline evaluated by contractile tension response was the same for both methods. It is concluded that the incubated ring preparation is better suited for release studies with the indirectly acting sympathomimetic amine tyramine. Furthermore, the use of the ring method is considered advantageous from a practical point of view since it allows for a better experimental design and yields higher amounts of collected tritium per ml.

The release of radioactively labelled transmitter substances from adrenergic neurones in blood vessels has been investigated both in vivo (Rosell et al. 1963) and in vitro (Su and Bevan 1967, 1970, Su 1968, Bevan et al. 1969, 1972, Häggendal et al. 1970, 1972, Hughes and Roth 1971, Allen et al. 1972).

The rabbit isolated pulmonary artery was considered a suitable organ for the study of release of noradrenaline (NA) because it is innervated only by postganglionic adrenergic and constrictor sympathetic fibers (Bevan and Su 1964, Verity and Bevan 1968). Furthermore, fundamental data concerning its morphology (Verity and Bevan 1968) and pharmacology (Bevan et al. 1970) is available.

Su and Bevan (1970) studied the release of transmitter from a helical strip of rabbit pulmonary artery by means of a superfusion technique (Su 1968). They loaded the vessel by incubating strips with tritium labelled noradrenaline (³H-NA)

which was then released by various procedures. The contractile response was measured simultaneously.

The purpose of the present work was to examine in detail the quantitative aspects of the ^{3}H NA release from the isolated pulmonary artery induced by either transmural stimulation or drugs. The preparation of a helical strip is probably associated with more surgical trauma to the tissue than a ring of the same vessel. This could conceivably interfere with the release of ^{3}H NA or other aspects of the sympathetic neuroeffector transmission process. We therefore compared the ^{3}H NA release and mechanical tension response of the superfused strip with that of an incubated ring preparation.

A preliminary report of this work was presented at the XXVI Meeting of the Scandinavian Pharmacological Society (Schrold and Nedergaard 1972).

Methods

Drugs. The following drugs listed with their molecular weights were used: Nicotine hydrogen tartrate (British Drug House Chemicals, Poole, England) 498; (—) noradrenaline hydrochloride, NA (Fluka, Buchs, Switzerland) 206; and tyramine hydrochloride, TA (British Drug House Chemicals, Poole, England) 174. The Radiochemical Centre (Amersham, England) supplied the following labelled compounds in a chromatographically pure form: (—) 7-^{3}H-noradrenaline hydrochloride, ^{3}H NA (specific activity 6.6 Ci/mmol) and 1,2-sidechain-^{3}H-tyramine hydrochloride, ^{3}H TA (specific activity 2 Ci/mmol). Appropriate solutions and dilutions of the sympathomimetic amines were prepared using HCl (0.01 N).

Salt solution. The concentration of ions in the physiological salt solution (PSS) was (in mmoles per liter): Na^+ 144.9, K^+ 4.9, Ca^{2+} 1.3, Mg^{2+} 1.2, Cl^- 126.7, HCO_3^- 25.0, SO_4^{2-} 1.2 and glucose 11.1. The solution contained also $CaNa_2EDTA$ (3×10^{-5} M) and ascorbic acid (1.1×10^{-4} M). The PSS was in equilibrium with O_2 containing 5% (v/v) CO_2 and had pH 7.4.

Preparations. Adult albino rabbits (1.8—2.3 kg b.wt.) were killed by cervical dislocation and bled rapidly from the carotid arteries. The main pulmonary artery was excised, cleaned of excess fat and paraarterial tissue and immersed in PSS.

Superfused strip. The method described by Su and Bevan (1970) was used. The pulmonary artery was cut spirally into a strip of 5×30 mm in size. The tissue weight was 42.81 ± 1.54 mg (mean ± S.E., N = 12). The strip was mounted vertically between a horizontal plastic rod and connected by a thread and a semirigid platinum wire (diameter 0.4 mm) to a transducer. The preparation was superfused with PSS prewarmed to 37° C at a constant rate (3.4 ml/min) by means of a peristaltic pump (LKB Varioperpex).

Incubated ring preparation. The pulmonary artery was divided in 2 almost equal sized rings. The width was approximately 4 mm and the weight 25.70 ± 1.00 mg (mean ± S.E., N = 35). Each vessel ring was mounted on a narrow horizontal supporting bar of an L-shaped plastic holder and connected by a semirigid platinum stirrup (diameter 0.4 mm) to a transducer. Each ring was placed in a jacketed bath filled with PSS (2.0 ml) maintained at 37° C (Fig. 1). The bath could automatically be emptied and filled with prewarmed (37° C) PSS at a constant time interval varying from 15 s to 60 min.

Stimulation. Both types of preparations were stimulated transmurally by a stimulator (Grass model S 4) in a standard manner (train of 300 biphasic pulses, pulse duration 0.3 ms, frequency 10 Hz, current 230 mA). This ensured electrode and supramaximal stimulation of the neuron terminals in the tissues. The current was monitored by display on a cathode ray oscilloscope (Advance Instruments, model OS 2000).

Recording. The isometric contractions of the smooth muscle in the artery was recorded by means of a sensitive force displacement transducer (Statham G 10 B ± 0.15 –350) connected to a linear pen recorder, either Servograph REC 51 (Radiometer, Copenhagen) or Sargent Welch, model SRG. With both preparations the tissue [illegible] stretched at 3 g tension for 10 min and subsequently maintained at [illegible] g resting tension. The preparation [illegible] allowed to equilibrate in the bath for at least 20 min before proceeding.

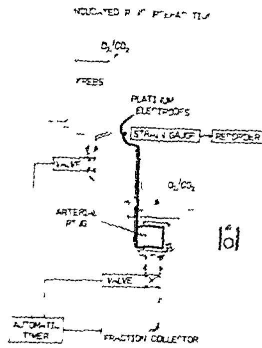


Fig 1 Schematic illustration of the incubated rabbit pulmonary artery ring preparation stimulated transmurally

Number of pulse and contraction height During an initial period (1 h) the incubated ring and superfused strip preparations were stimulated with the standard stimulus (vide supra) 3–4 times at 10 min intervals until the response to stimulation increased by less than 10 % of the previous one. The relationship between number of pulses and elicited contraction height was then studied by increasing the number of pulses (10–3000). The interval between stimulation was 10 min

NA cumulative concentration response curves Logarithmically spaced concentrations 10^{-9}–3×10^{-4} M of (—) NA were added to either the bath or superfusion fluid

Tritium release

Strip preparation This preparation was initially immersed in PSS (6 ml) maintained at 37 C. To this medium was added ^{3}H NA (8 × 10 M). After 1 h the organ bath was emptied and non radioactive PSS previously warmed to 37 C and equilibrated with the O_2 CO_2 mixture was allowed to superfuse the strip at a constant rate (3 4 ml/min). The perfusate dripped directly into a tube (10 0 ml) placed in a fraction collector (LKB Ultrorac 000). The tube was changed every 2 min

Ring preparation The ring was incubated in PSS (2 ml) with ^{3}H NA (8 × 10 M) for 1 h. The bath was then emptied and refilled automatically with PSS (Fig 1). The fractions (ml) were collected every min. Preliminary experiments (N = 3) demonstrated that the total ^{3}H release induced by FS was considerably lower when the fraction collection period was extended from 2 to 10 min. This may reflect in part an increased re uptake by adrenergic neurones of released ^{3}H NA from the extracellular compartment during the longer collection period

Retention of ^{3}H TA Essentially the method described by Nedergaard et al (1) was used. Rings and strips of pulmonary artery were incubated (2 0 ml) and superfused (3 4 ml/min) respectively with ^{3}H TA (10 M 0 2 μCi/ml bath) for 10 min. Then the tissues were placed in PSS (6 ml) for 10 min in order to remove non-specifically bound ^{3}H TA. After removal of the tissue from the bath the rings were cut open into a rectangle. Rings and strips were blotted under pressure (50 g) on filter paper (Munktell's no 8) previously moistened with the salt solution in a standard manner and weighed (range 1 –6 mg). Each sample was transferred to the bottom of a 25 ml polyethylene liquid scintillation counting vial (Scintex Denmark)

Tritium determination ^{3}H TA uptake After addition of 0 50 ml Soluene (Packard Instruments Company) the closed vials stood for 10 h to allow for partial digestion of the tissue. T

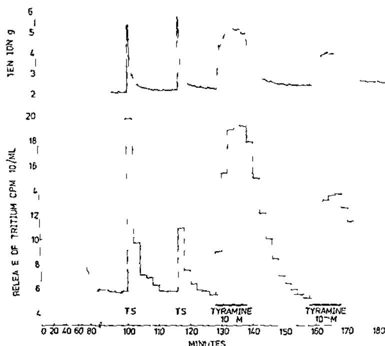


Fig 2 Tritium release a d contractile response from a typical experiment with an incubated rabbit pulmonary artery ring *in vitro* Abscissa Time (min) after an initial loading period (60 min) with ^{3}H NA (8 × 10 7 M) Ordinate Upper graph Smooth muscle tension (g) The tracing illustrates the orig nal record ng of mechanical activity Lo er graph Release of tritium (cpm × 10 /ml) Fractions (2 ml) were collected every 2 min Fraction content of radioactivity is shown only for every fifth collected during the initial spontaneous efflux period (90 min) The preparation (24 5 mg) was stimulated transmurally (TS) Tyramine (10 4 M) was maintained constant in the bath for 10 min The horizontal bars indicate the duration of stimu lation and tyramine addition The area under the stipled lines represents the presumed passi e release

each vial was add d 18 ml of a fluor solution with the followi g compo tion I iqu fluor (Packard) 42 ml absolute methanol reagent grade 300 ml and toluene reagent grade to 1 liter

H r lease Aliquots (1 00 ml) of the collected fractions were added to 10 ml of fluor solu tion (Bray 1960) Radioactivity was measured w th a l q uid scintillation spectrometer (Beckman LS 250) The counting efficiency was 18 % as det rm ned by adding a standard of ^{3}H water to the fluor solution The quench ng produ ed b the aliquots were constant for all fract ons The background was 25 cpm The amou t of rel ased trit um per fractio i is expressed as radio-activity present in each ml fraction (cpm/ml r dpm/ml) In some cases the rel a e was cor rected for passive release The latt r was cal ul ted by subt acti g from each relea s lue the mean value from the t o fractions obtained just prior to stimulat on or drug addit on Total amount of release n a p ak as calculated by ummation of ea h fract on al ie wh h ntered in the formation of the peak The sum was corrected for passi e relea e tissue weight counting efficiency pecific a t vit and fraction volume and expressed as pmoles/g A RC 7000 Basic Computer (A/S R gnecentralen Copenh g n) wa used n these calculat ons

T it um conte t At th end of each release e periment the ^{3}H-content of the ti ue was assayed From this al e and the measured prev ou s fract onal relea es th amount of trit um pre e t in the t ssu at any given time could be calcul ted appropriately

Stat t cal a aly s Stud nt t test was used

TABLE I Total tritium release elicited by transmural stimulation, tyramine and nicotine on incubated ring or superfused strip preparations of rabbit pulmonary artery *in vitro*. After an initial loading period (60 min) with ³H-NA (8×10⁻⁸ M) and a wash-out period (100 min) the tissue was subjected to transmural stimulation and/or drugs. The total tritium release elicited by these treatments was corrected for passive efflux. Transmural stimulation and tyramine treatments were done on the same artery in the order as indicated; nicotine addition was carried out on separate tissue. Data represent mean values±S.E. Figures in brackets indicate number of experiments

Treatment	No	Time[a] (min)	³H release		Calculated ³H-content in the tissue[b]	
			Incubated ring	Superfused strip	Incubated ring	Superfused strip
			pmoles/g			
Transmural	I	100	62±10 (7)	67±11 (4)	3396±376 (7)	3099±158 (4)
Stimulation	II	114	24± 4 (7)	32± 7 (4)	3204±362 (7)	2874±159 (4)
Tyramine	III	128	369±49[d] (6)	136±15 (4)	3171±382 (6)	2693±162 (4)
(10⁻ M)	IV	158	174±30[d] (3)	77±19 (3)	2403±692 (3)	2512±125 (3)
Nicotine	I	100	68±12 (5)	70±20 (6)	2544±205 (5)	3814±420 (6)
(10⁻ M)						

a Time after commencement of wash out
b The ³H content in the tissue immediately preceding treatment. For calculation see Methods
c Concentration maintained constant for 10 min in the bath
d Differs ($p < 0.05$) from superfused strip (same horizontal column)

Results

A Tritium release

The passive outflow of tritium from the artery ring (Fig. 2) and strip to the incubation and superfusion fluid respectively declined rapidly during the initial period. 90 min after the commencement of the wash out period the outflow reached an almost steady level for both the incubated ring (600 cpm/ml) and superfused strip (400 cpm/ml).

1 Transmural stimulation. The primary data are examplified in Fig. 3. Upon initiation of stimulation the artery ring preparation began to develop tension within 5 s. The tension rose during the 30 s period of stimulation and continued several seconds after termination of stimulation. Relaxation rapidly ensued and was complete in the course of the subsequent 15 min. Repeated stimulation caused a contraction of the same height.

Stimulation also caused a sharp elevation in the ³H release from the tissue into the bath to as high as 400 % of the prestimulation level. A subsequent stimulation was associated with a decline in ³H release. Neurogenic stimulation of the superfused strip preparation caused a similar pattern of response with regard to both tension development and ³H release.

The amount of radioactivity present in each fraction following stimulation of the incubated ring was up to 3 times higher than that seen with the strip. However the total amount of released tritium did not differ between the two preparations (Table I).

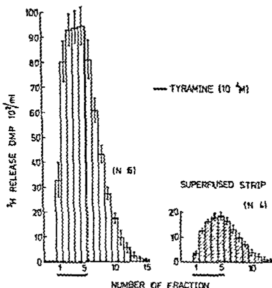


Fig 3 Comparison between incubated rings and superfused strips of rabbit pulmonary artery in vitro with regard to tyramine induced tritium release Ordinate Mean tritium release (dpm $\times 10^{-3}$/ml) in each fraction (2 ml) The values are corrected for passive release Abscissa Number of fractions collected every 2 min The horizontal bars indicate the contact time for tyramine (10^{-4} M) with the preparation The vertical bars represent the ± S E

2 *Tyramine* Fig 2 illustrates the primary data for the effect of TA on mechanical tension response and ³H NA release After a latency period (15—20 s) TA (10^{-4} M) elicited a contraction where the tension rose continuously during the initial part (4—7 min) of the exposure period (10 min) Following wash out of TA the relaxation of the vessel was complete about 15 min later

The concomitant release of total tritium into the bath fluid rose to as high as 510 % of predrug level A subsequent addition of TA (10^{-4} M) caused development of tachyphylaxis to both ³H release and contractile response

Essentially the same type of response was seen with TA (10^{-4} M) on the superfused strip preparation

A comparison of the two methods as regards the initial 10 fractions showed that the amount of tritium present in each fraction was from 9—13 times higher for the incubated ring than the superfused strip (Fig 3) This reflects in part the higher sensitivity of the former method

The total amount of TA induced ³H release from the incubated ring was 270 % of that seen with the strip (Table I) On the other hand the mean ³H-content of the tissue just prior to stimulation also did not differ (Table I)

3 *Nicotine* Nicotine (10^{-4} M) caused a transient contractile response of the artery with either method Relaxation of the smooth muscle was complete even before nicotine was washed out The ³H release followed a similar time course

The amount of radioactivity present in the initial 5 fractions from incubated rings

C Receptor s nsitivity to NA

The contractile tension elicited by cumulative addition of (—) NA (10^{-9}—3×10^{-4} M) was higher with incubated rings than superfused strips (Fig 4) However a per cent plot of the data yielded curves with similar slopes (Fig 4) which indicates that the sensitivity of the receptors towards exogenous NA is the same for both types of preparation

Similar results were obtained also for TS with regard to number of pulses (10—3000) delivered (Fig 4) The ED_{50} was 220 and 230 pulses for the superfused strip and incubated ring respectively

D Effect of bretylium on neurogenic response

Bevan and Su (1970) found that transmural stimulation during superfusion produced selective and maximal activation of the nervous elements not achieved when the tissue was in a bath (Su 1968) In order to examine this aspect we studied the effect of bretylium an adrenergic neurone blocking agent on the neurogenic tension response

Bretylium (10 M) completely blocked the response of incubated rings to TS Using the same technique bretylium (10 M) neither moved the concentration response curve of NA (10^{-9}—3×10^{-4} M) nor decreased the maximal tension response (3 expts)

Discussion

The release of ³H NA from adrenergic neurones and contractions of smooth muscle of isolated pulmonary artery can be measured simultaneously by the technique of combining TS (Paterson 1965) with either superfused strips (Su and Bevan 1970) or rings submerged in fluid in a small bath (incubated rings)

The present results indicate that the use of incubated rings of pulmonary artery rather than superfused strips (Bevan and Su 1970) offers several advantages With the former method the use of 2 arterial rings derived from the same rabbit allows for a better experimental design with the possibility of carrying out test and control experiments in parallel The superfused strip method on the other hand required all available arterial tissue for a single helical strip Dividing the strip into 2 equal sized lengths was not considered practical as this would severely decrease the actual amount of tritium (cpm/ml) in each collected fraction and thereby hamper the accuracy of ³H determination Furthermore the mechanical tension response would be reduced

Using the same stimulation parameters as Bevan and Su (1970) we found that TS during both incubation of pulmonary artery rings produced selective and maximal stimulation of the adrenergic neurone This was indicated by the finding that bretylium in a concentration which did not decrease the mechanical contraction response to exogenous NA completely blocked the neurogenic contractions

The use of superfused strips in a study of the effect of ³H NA and possibly other indirectly acting sympathomimetic amines is considered less suitable for this purpose

than incubated rings. This is suggested by the finding that TA released totally only one third of tritium with the former method compared to the latter (Table I). To explain this difference between the two methods 3 possibilities may be considered: (1) The access of exogenous TA to the adrenergic neurones might be impaired in the superfused strips; (2) the characteristics of the release and diffusion of ^{3}H NA from the nerve terminals at the adventitio-medial junction to the outside of the tissue might be different; and (3) the ^{3}H amount present in the superfused respectively incubated tissue might differ at the moment of TA application. The first possibility is ruled out by the observed higher retention of ^{3}H TA seen with superfused strips (Table II) and the second is not considered likely as the total release of tritium after both TS and nicotine was the same for both methods (Table I).

Likewise since the ^{3}H-content in the tissue did not differ (Table I) the third possibility is *a priori* unlikely. However, it may be that the total ^{3}H-content does not adequately describe the size of the TA sensitive pool. Alternatively, we infer by the principle of exclusion that the release mechanism of this pool has been damaged possibly due to excess surgical trauma inherent to the preparation of a helical strip.

Another advantage of the incubated ring method is that the functional integrity of the tissue is maintained better when arterial rings are used rather than helical strips. This was reflected in the higher mechanical tension responses seen with exogenous NA and TS (Fig. 4). However, the receptor sensitivity to NA of either exogenous or endogenous origin was the same for both methods as indicated by a per cent plot of the primary data (Fig. 4).

No attempt was made in the present work to determine the nature of the released ^{3}H material as this was not considered germane to the primary aims of our study. According to Su and Bevan (1970) only one third of the spontaneously released tritium from superfused strips of rabbit pulmonary artery was ^{3}H NA. However, when they subjected the preparation to TS, ^{3}H NA accounted for 90% of the ^{3}H release.

The ^{3}H release induced by either TS or drugs may serve as an indicator of the neural release of endogenous NA. Just how representative the newly bound ^{3}H NA of exogenous origin would be of the total transmitter stores is uncertain (Bevan *et al.* 1970). This may especially be the case with regard to the storage pool from which ^{3}H NA is released by TS. Thus, the first stimulation (Fig. 2, Table I) caused a great ^{3}H release compared to the subsequent stimulation, while the contractile responses remained the same (Su and Bevan 1967, present work Fig. 2). It is unlikely that the first ^{3}H-output was primarily due to ^{3}H NA present in the extracellular space since such an initial disproportionately large ^{3}H release was not seen when ^{3}H metaraminol was used in place of ^{3}H NA (Schrold and Nedergaard, unpublished).

The observed dissociation between ^{3}H release and contraction response could be due to an enhanced release of newly synthesized NA in the second stimulation period relative to the first. This possibility is seemingly rendered unlikely, since activation of sympathetic neurones in isolated pulmonary artery primarily released endogenous NA rather than newly synthesized amine (Kupferman *et al.* 1970). However, the

stimulation parameters used in their study and ours were not the same Since the relative importance of new synthesis in maintaining transmitter release may vary with the interval and rate of stimulation (Kopin *et al* 1968) the findings of Kupferman *et al* (1970) may possibly not be applicable to the present work Thus with regard to TS the newly bound ³H NA may serve more as a qualitative indicator of endogenous NA while the labelled amine may possibly reflect more quantitatively the events in the TA sensitive storage pool This is suggested by the concomitant decrease in both ³H release and contraction response seen with TA (Fig 2)

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References

ALLEN G S M J RAND and D F STORY The *dextro* i omer of no adrenaline as a false transmitter *J Pharm Pha ma ol* 19?? 24 335—336

BEVAN J A and C SU The sympathetic mechanism in the isolated pulmonary artery of the rabbit *B t J Pharmacol* 1964 ?? 176—18?

BEVAN J A G B CHESHER and C SU Release of adrenergic transmitter from terminal nerve plexus in artery *Agents and A tions* 1969 *1* 20—?6

BEVAN J A O A NEDERGAARD J V O HER C SU J TOROK and M VERITY On the mechani m of neuromuscular transmission in blood ves els *Pro Fou th Int Cong Pha ma ol* 1970 7—?3

BEVAN J A R D BEVAN R E PURDY C P ROBINSON C SU and J G WATERSON Comparison of adrenergic mechanisms in an elast c and a muscular artery of the rabbit *C rculat R s* 197? *30* 541—548

BRAY G A A simple efficient liqu d scintillator for counting aqueous solutions in a l quid cintillation counter *Analyt Biochem* 1960 *1* ?79—28?

HAGGENDAL J B JOHANSSON J JONASON and B LJUNG Cor elation between noradrenaline rel a e and e fector espon e to nerve stimulation in rat portal ve n in itro *A a Physiol s and* 19 0 Suppl 34? 17—3?

HAGGENDAL J B JOHANSSON J JONASON and B LJUNG Effects of phenoxybenzamine on transmitter release and effector respons in isolated po tal vein *J Pha m Pha macol* 19?? 24 ?61—164

HUGHES J and R H ROTH Evidence that angiotensin enhances t ansmitter release during mpathetic nerve stimul t on *B t J Pharma ol* 1971 *41* ?39—?5?

KOPIN I J G P PREESE K R KRAUSS and V K WEISE Selective release of new l yn thesized norepinephrine from the rat spleen du ing ympathetic nerve timulation *J Pha ma l p Th* 1968 *161* 271—??8

KUPFERMAN A C N GILLIS and R H ROTH Influence of sympathet c ne st m l tion on con ersion of H³ ty osine to H³-catecholamine and on H norep neph in di po i on in abbit pulmonary artery *J Pha ma ol xp Th* 19 0 *17?* ?14—???

NEDERGAARD O A A V ONE and J A BEVAN Effect of the chelati g ag nts EDTA ? b pyrid ne 8 hydroxyquinoline and py ophosphoric acid on no epinephrin upt ke b r b t ao ta *J Pha macol xp Ther* 1968 *163* 136—146

P TERSON G The response to tran m ral stimulation of solated a terial t p and i modif tion by drugs *J Pha m Pha mac l* 1965 *17* 341—349

P SELL S I J KOPIN and J AXELROD Fate of H³ no d enal e i kel tal mus le bef and following ympathet c stim lation *Am J Phy l* ? 63 31 ? 1

SEHROLD J and O A NEDERGAARD Release of ³H n d al n (i m b t d and u e fu ed abo t pulmonary artery *A ta pha ma l Kbh* ? ?? *Suppl* ? 5 63

S C A superfused and transmu all timul t d art y pa i n *P* *H i n P a na l So* 1968 *11* 143—144

SU C a d J A BEVAN Release of ³H no d e a fr n aso ustricto erve *J Pha m Pla ma ol* 1967 *19* 6?5—6 6

SU C a d J A BEVAN The release of H³ n l n in art rial strips st d ed by the tech nque of superfusion and t an mu al st m l *J Pha ma l exp Th* 19 0 *17?* 6 58

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An Autoradiographic Study of the Intestinal Absorption of Palmitic and Oleic Acid

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Abstract

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The distribution of intraluminally incubated long chain fatty acids (LFA ^{3}H-palmitic acid, ^{3}H-oleic acid) within the intestinal villi were studied during in vivo conditions with an autoradiographic technique which prevented tracer losses from the tissue The results show that LFA are fairly diffusely localized throughout the whole epithelial cell and to some extent also in the lamina propria after 1 or 10 min incubation Intense vasodilatation or a albumin solution administered intraluminally after the tracer incubation is able to extract a large amount of the absorbed LFA from the epithelial cells These observations strongly suggest that LFA enter the epithelial cells faster than they are re-esterified into triglycerides resulting in a pool of diffusible LFA within the cells Although the cells are in close contact with the subepithelial capillary network and subsequent rapid absorption via the blood stream does not occur It is proposed that the hindrance to net blood absorption of the lipid soluble LFA is a "short circuiting" of the easily diffusible LFA in the mucosal countercurrent exchanger

It is generally agreed that in mammals the short chain fatty acids (i e fatty acids with less than 10—12 carbon atoms) are absorbed from the small intestine mainly via the blood while the long chain fatty acids are predominantly transported via the lymph as triglycerides in chylomicrons (see e g Brindley and Hubscher 1966 Hyun Vahouny and Treadwell 1967) The factors determining this partition between blood and lymph are largely unknown (Dawson 1967) although it is usually explained in terms of the rapid esterification of the long chain fatty acids in the apical parts of the epithelial cells while this should not be the case with the short chain fatty acids Thus this rapid esterification is supposed to hinder the long chain fatty acids from reaching the subepithelial capillary network while the short ones would diffuse freely through the epithelial cells to the blood stream

In recent experiments (Lundgren 1967) it was demonstrated that a countercurrent exchange mechanism exists in the mucosa of the small intestine of the cat The countercurrent exchange is supposed to take place mainly between the small non

branching arterial vessels in central parts of the villi and the surrounding subepithelial capillary network Such a mechanism would tend to delay the net ab orption of particularly lipid soluble substances due to short-circuiting of these solutes in the countercurrent exchanger *i e* the absorbed easily diffusible solute diffuses along a concentration gradient from the subepithelial capillaries to the central arterial vessel Water soluble substances on the other hand being pore restricted with respect to their capillary passage are less easily trapped in the exchanger Thus it was demonstrated that lipophilic solutes such as oxygen krypton and antipyrine are readily trapped in the exchanger while hydrophilic substances such as urea and rubidium are less affected by the countercurrent mechanism (Lundgren 1967) — The present autoradiographic study was undertaken to test whether this type of mechanism could be of any importance in explaining the different routes of absorp tion of fatty acids since it is well known that their lipid olubility decreases with decreasing length of the acid

Methods

A Operative procedures and dete mination of blood flow The observations were carried out on 25 cats anesthetized with chloralose (50—70 mg b wt) after induction with ether The ats had been deprived of food for at least 24 h and had no ob ious signs of intestinal infection

After tracheotomy the abdomen was opened in the midl ne and the greater omentum and the spleen extirpated The experiments were performed on 3—4 isolated intestinal segments 3—5 cm long from the proximal jejunum the remainder of the intestinal tract being extirpated The lumen of the segments was rinsed with body warm saline

After heparinizing the animal (3—5 mg/kg b t) blood pressure was measured in the right femoral artery by a mercury manom ter or a pressure transducer (Statham P 23 AC) The superior mesenteric vein draining all the intestinal egments and their lymph nodes was cannulated and connected to an optical drop recorder—ordinate writer unit recording either on a kymograph or on a Grass polygraph The venous blood was returned to the animal *via* a funnel connected to a catheter in the right jugular vein

All the splanchni nerves were regularly cut while the parasympathetic supply to the small intestine was left intact. To induce vasodilatation constant infusions of isopropylnoradrenaline 5—10 μg/min were made through a side branch of the superior mesenteric artery

B Rad ochemicals The absorption of palmitic acid 9 10-^{3}H and oleic acid 9 10-^{3}H (the Radiochemical Centre Amersham England) was stud ed by autoradiography the tracers being admin stered into the lumen of the i olated intestinal segments as follows

Palmit c acid The labelled compound (purity according to producer > 97 per cent) was del vered from the manufacturer dissol ed in hexene benzene (50 50) 500 μCi (specific activity 500 mCi/mmol) was put in an Erle meyer flask and the solvent e aporated under nit ogen after which the labelled palmitic acid was emulsified in 1 ml of bile (undiluted gall bladder bile or bile d luted 5—10 times with saline) or in 1 ml of T een 20 (50 mg/ml saline) After shaking the clear solution for 2—3 h in a bath at 37 C 0 2—0 5 ml was adm nistered into the intestinal lumen wh ch had usually been pre ncubated for 1/2—2 h w th unlabelled palmatic acid emulsified in bile pH of the administered solutions measured 6 8—7 5

Oleic acid The labelled sub tance (purity according to manufacturer 99 per cent) delivered dissol ed in benzene was treated as described above fo palm tic a d 200 Ci (pecif activit 4 9 Ci/mmol) emulsified in 1 ml b le (gall bladde b l d luted fi e times with saline) was placed in the lumen of an isolated intestinal segment

C Auto ad og aphic t chnique After administrati n of the tracer olutio s described above into the lumen of the different intestinal segments the wer at a p ed termined tim flu hed with saline and immed ately frozen and extirpated In t ea l e pe iments the segm nts were first extirpated and then immediately fro en in a dry i e ac t n m xture Later they were promptly frozen *in situ* by means of 2 specially sh ped blocks of alum nium that had been precooled in liqu d nit ogen extirpated and put in liquid nitr ge the ent re procedure lasting some 5 ec

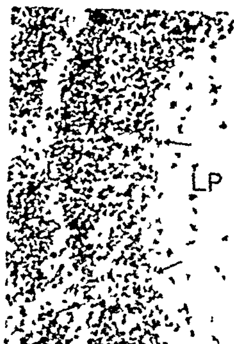


Fig. 1. The distribution of ^{3}H palmitic acid within the epithelial cells and the lamina propria close to a villous tip after 1 min intraluminal incubation of a bile ^{3}H palmitic acid solution. Arrow indicates epithelial cell borders. LP = lamina propria. L = luminal gap between two adjacent villi. Exposure time 42 days. Toluidine blue. 1000 ×

Glass slides cleaned in 95 % ethanol and in a Na_2CO_3–H_2SO_4 solution were covered by a thin layer of gelatin and chrome alum solution (Rogers 1967, p. 211). A liquid emulsion of Ilford K2 or K5 was diluted with distilled water (50:50) and smeared onto the glass slides in a dark room at 35 °C and 80 % relative humidity, after which they were allowed to dry in vertical position. The slides were then stored at 4 °C until used (maximally 4–6 weeks). The emulsion [illegible] and a thickness similar to that reported by Kopriwa and Leblond (1962) (J. Sjöstrand, personal communication).

The frozen intestinal segments were cut transversely into 5 or 10 µm thick sections in a cryostat (Dittes Duppa) kept between −20 °C and −25 °C in dark room. The tissue sections were adhered to the emulsion-coated glass slides in three different ways. In early experiments they were thawed onto the glass slide by the finger tip, which means a possible risk of diffusion artifacts. This thawing was therefore avoided in later experiments: instead the glass slides were either made sticky by dipping them in 5 % glycerol dissolved in waterfree ethanol (Hammarström, Appelgren and Ullberg 1965), or the method described by Appleton (1964) was used. In the latter method the glass slides were warmed to −5 °C on a small thermoelement inside the cryostat, after which the tissue sections were adhered to the slides with a light touch. The results obtained with the three different techniques were similar.

The autoradiographs were exposed at −20 °C together with a drying agent (Silica gel) for varying periods of time (4–6 weeks), the time of exposure depending on the amount of activity in the intestinal segments. Before developing the autoradiographs they were allowed to equilibrate with room temperature for 1 h. The tissue sections were then fixed in 5 % acetic ethanol, developed in Phen X® (Ilford) for 5 min, fixed for 5 min in Ilofix® (Ilford) and rinsed in distilled water. The sections were stained in 1 % toluidine blue at pH 4 or hematoxyline-eosin and mounted in DePex. Control non-radioactive tissue sections gave no chemographic effects on the emulsion with the technique used.

Results

The solutions containing the labelled fatty acid were incubated in the lumen of the small intestine during *in vivo* conditions for 1 or 20 min. The autoradiographs obtained with the two long chain fatty acids will be described separately below.



Fig 2 The distribution of ^{3}H palmitic acid within the villous tissue at a villous tip (left panel) and at a villous base (right panel) after 1 min intraluminal incubation of a bile ^{3}H palmitic acid solution Arrows indicate the border between the epithelial cells and the lamina propria C = border between the villus and the crypt L = luminal gap between adjacent villi LP = lamina propria Exposure time 4? days Toluidine blue 180 ×

A Palmitic acid

Fig 1 illustrates an autoradiograph of intestinal epithelial cells close to the tips of a villus from an experiment in which a bile solution containing ^{3}H palmitic acid was incubated for only 1 min in the intestinal lumen Arrows indicate the boundary between the epithelial cells and the core of the villus This autoradiograph shows that the blackening is more or less diffusely distributed throughout the epithelial cells with a more heavy graining in the apical parts of most of the cells A certain number of grains are also found in the villous core It is thus clear that the labelled compound had in significant amounts reached the basal parts of the epithelial cells and al o to some extent the lamina propria

In order to more clearly illustrate the di tribution of the labelled compound along the length of the villus Fig 2 hows autoradiographs from the same experiment as Fig 1 but at a lower magnification It is evident that the labelling is most heavy in the epithelial cells of the tip portion of the villus

Fig 3 illustrates the distribution of grains in the villus in an experiment in ^{3}H palmitic acid in oxygenated bile had been incubated intraluminally f



Fig. 3. The distribution of absorbed ^{3}H palmitic acid along the villous length during intestinal ischemia, the left panel showing the villous tip, the right panel the villous base. An intestinal segment was incubated for 1 min with an oxygenated bile solution containing ^{3}H palmitic acid. Arrows indicate the border between epithelial cells and lamina propria. C = border between the villus and the crypt. I = luminal gap between adjacent villi. LP = lamina propria. Exposure time 33 days. Hematoxylin-eosin. Phase contrast microscopy 230 ×.

but during intestinal ischemia produced by prior clamping of the cognate vessels. The left panel shows the tip of a villus while its base is seen in the right panel. The grain distribution between epithelial cells and lamina propria is similar to that illustrated in Fig. 1 and 2 but the grain distribution along the villous length is far more even when villous blood supply had been stopped. Evidently entrance of ^{3}H palmitic into the epithelial cells takes place along the entire villous length.

The autoradiographic picture seen after 20 min tracer incubation was somewhat different from that observed after 1 min incubation, as shown in Fig. 4. The blackening of the cells is now more uneven, being more heavy in their apical parts where the triglyceride reesterification takes place. The distribution of radioactivity along the villous length after 20 min intraluminal incubation of ^{3}H palmitic acid in bile is shown in Fig. 5, the blackening being now concentrated to the villous tip as a cap.

The same picture as in Fig. 1, 2, 4 and 5 was seen when the intestinal segment had not been "preloaded" with unlabelled palmitic acid for 1/2–2 h as well as in experiments in which Tween 20 instead of bile was used as the intraluminal emulsifier for palmitic acid.

Obviously no conclusions can be drawn from these autoradiographic experiments concerning the chemical form in which the labelled compound is present in the epithelial cells. However, in order to examine whether the labelled compound in the villi still existed in a "freely" diffusible form also after 20 min incubation, experiments were performed in which 1 ml of a 7 per cent solution of human albumin was placed in the intestinal lumen for 1 min after 20 min incubation period. To remove the labelled bile solution from the lumen, the albumin incubation was preceded by a rapid flushing of the intestinal lumen with 100 ml bodywarm saline, a procedure which in control experiments did not change the autoradiographic picture seen in Fig. 4. Fig. 6 illustrates an autoradiograph from an albumin experiment. The



Fig 4

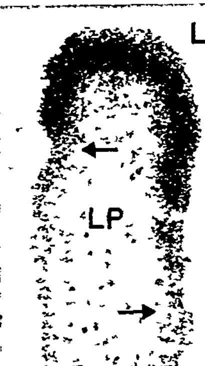


Fig 5

Fig 4 The distribution of ^3H palmitic acid within the epithelial cells and the lamina propria close to a villous tip after 20 min intraluminal incubation of a bile-solution containing ^3H palmitic acid. Arrow indicates the border between epithelial cells and lamina propria. L = lumen LP = lamina propria n = nucleus of an epithelial cell. Exposure time 35 days. Hematoxyline-eosin 1120 ×

Fig 5 The distribution of ^3H palmitic acid within the upper 1/3 of a villus after 20 min intraluminal incubation of a bile solution containing ^3H palmitic acid Arrows indicate the border between epithelial cells and lamina propria. L = lumen LP = lamina propria. Exposure time 35 days. Hematoxyline-eosin 345 ×

blackening is now concentrated in rounded structures just above the nucleus (n) the rest of the cell and the lamina propria being uniformly but much less heavily grained Comparing Fig 4 and 6 it seems justified to conclude that a substantial fraction of the absorbed fatty acid still exists in a diffusible form within the cell even after 20 min incubation and may be "extracted" by means of an albumin solution in the gut lumen

In one experiment performed as described above bile instead of an albumin solution was flushed through the intestinal lumen The appearance of the autoradiogram in that experiment was similar to one illustrated in Fig 5 although the rounded structures were not so prominent

In three other experiments the intestinal lumen was incubated with ^3H palmitic acid for 20 min and then flushed with 100 ml of a physiological saline solut



Fig. 6. The distribution of ³H palmitic acid within the epithelial cells and the lamina propria after [illegible]0 min intraluminal incubation of a bile solution containing ³H palmitic acid followed by a 3 min incubation of a 7 % a[illegible] solution. Arrows indicate the epithelial cell border. L = lumen. LP = lamina propria. n = nucleus of an epithelial cell. Exposure time 35 days. Hematoxyline-eosin. 1000 ×.

remove the labelled bile solution. Five minutes later the segment, which throughout had had a normal blood supply, was extirpated, frozen and treated as described in Methods. The autoradiographic picture of the intestinal cells was similar to that illustrated in Fig. 4 and 5. Thus, the albumin of the cat's own blood, when circulating along the inner "basal" surface of the epithelial cells, seemed unable to remove the labelled compound from the intestinal cells, at least to judge from these semi-quantitative autoradiographic experiments.

The experiments described so far were all performed during "resting" blood flow conditions, *i.e.* venous outflow amounted to 20—40 ml/min × 100 g tissue. *In five experiments a 4—5 fold flow increase was maintained for 20 min by a constant intraarterial infusion of isopropylnoradrenaline. The intracellular distribution of radioactive tracer was in most of these experiments similar to that illustrated in Fig. 6. Thus, the blackening was concentrated in rounded structures lying just apically to the nucleus.*

B. Oleic acid

In two experiments autoradiograms were made after intestinal incubation with ³H oleic acid in bile for 1 or 20 min at "resting" blood flow. Autoradiograms were made on intestinal segments extirpated during "control" conditions, after *intraluminal* albumin extraction following a 20 min incubation of the labelled solution (*cf.* Fig. 6) as well as 5 min after flushing the intestinal lumen with a large volume of saline. In all these different types of experiments the autoradiographic picture was similar to that described above for ³H palmitic acid.

Discussion

The present study was performed during *in vivo* conditions in order to study by autoradiography the distribution within the villi of labelled long chain fatty acids after various times of exposing the lumen to the labelled acid Such studies have been performed earlier by several authors (Strauss and Ito 1965 Jersild 1966 Dermer 1968 Strauss 1968 Carlier 1971) However most of these studies were performed with an autoradiographic technique at the electronmicroscopic level the preparation of which extracted up to 40 per cent of the tissue content of the absorbed lipid material (Carlier 1968 Dermer 1968 Saunders Wilson and Rubin 1968 Buschman and Taylor 1971) Furthermore most of these studies were carried out during *in vitro* conditions without any villous blood supply and with the aim of investigating the intracellular distribution of the tracer material. The present study was performed with an autoradiographic technique where fresh tissue material without any histo-chemical treatment preventing tracer losses from the tissue This approach limited the resolution power to the light microscopy level but this study was primarily aimed at exploring the distribution of the labelled material within the villus as whole However the technique still allowed a fairly accurate intracellular localisation since the resolution power amounted to 1—2 μm with the emulsion thickness used (Appelton 1966 Rogers 1969)

According to the generally accepted hypothesis long chain fatty acids enter the epithelial cells by diffusion but are rapidly esterified in their apical parts while short chain fatty acids diffuse freely through the epithelial cells to reach the blood capillaries Thus the partition between lymph and blood would be primarily dependent on the specificity and capacity of the re-esterifying enzymes in the intestinal epithelial cells (*cf* Gelb and Kessler 1963 Brindley and Hubscher 1966) This hypothesis then implies that the absorbed long chain fatty acids would be unevenly distributed within the epithelial cell and that they would in only a very small fraction reach beyond the apical cell parts to enter the capillaries However this seems not to be the case as illustrated in Fig 1 which shows a fairly diffuse graining of the entire epithelial cell

Furthermore according to the generally accepted hypothesis a comparatively small fraction of the absorbed fatty acids would exist in the cell in the form of free fatty acids since the enzymatic re-esterifying capacity is assumed to be so high as to prevent the fatty acid from reaching the capillaries Observations made in the present study and in several other investigations using biochemical techniques argue strongly against this view In the present study it was demonstrated (Fig 4 and 6) that an albumin solution placed in the intestinal lumen was able to extract some of the intra-epithelial fatty acid demonstrating that this tracer after 20 min still existed in a diffusible form probably as free fatty acid Observations made in several other studies corroborate this view in a more quantitative manner Most authors report that 20—30 per cent of the absorbed long chain fatty acids exist as free fatty acids even after 20—30 min incubation (*in vitro* Gordon and Kern Webb Hamilton and Dawson 1969 Hoffman 1970 Bennett Clark 1971

Hamilton 1971) and some even higher values around 50 per cent (Cather 1971). Moreover, Johnston (1959) found that 13 per cent of the labelled fatty acids were transferred to the serosal surface of an everted small intestine.

All the abovementioned observations strongly suggest that the esterifying enzymes in the supranuclear part of the epithelial cell cannot by far re-esterify the labelled palmitic acid at the same rate as it enters the epithelial cell. Further, in vitro experiments suggest that a maximal limit of triglyceride formation is present (Clark, Lanz and Senior 1969, Hoffman and Simmonds 1971, Hamilton 1971).

It should be underlined that the diffusion of solutes in water for distance of 20–25 μm, corresponding to the height of an epithelial cell, is extremely rapid. It can for example be calculated that sucrose (molecular weight 342, diffusion constant 37° C 0.70 × 10 cm²/sec), a molecule of the same size as palmitic and oleic acids, reaches a 50 per cent equilibrium across a 25 μm thick "sheet" of water in 0.04 sec (see Dawson 1970). Assuming that diffusion of free fatty acid molecules occurs into the cell, these calculations would indicate that the long chain fatty acid would reach the basal parts of the cell very quickly indeed. This is also corroborated by unpublished observations (Jodal and Lundgren) that revealed radioactivity in the venous effluent from an intestinal segment within seconds after placing ³H palmitic acid in bile in the gut lumen. Furthermore, it was demonstrated by Hamilton (1971) that diffusion into the cell of ¹⁴C-labelled oleic acid in vivo occurs very rapidly, about 20 per cent of the total radioactivity administered intraluminally being found in the mucosa after only a few seconds.

The different routes of intestinal absorption for long chain and short chain fatty acid might be explained by a relative impermeability of the epithelial cell membrane facing the capillary. In fact, some autoradiographs presented here (see e.g. Fig. 1, 2 and 3) may favour such a view, the graining in the villous core being low compared to the intracellular compartment. However, it seems a priori unlikely that the lipid cell membrane would be able to hinder net transport of such extremely lipid soluble solutes as long chain fatty acids, the more so since the "mucosal side" of the cell membrane easily allows fatty acids to pass, as also shown by Hamilton (1971) and discussed above. Further, the rapid appearance of radioactivity in the venous effluent after intraluminal incubation of labelled palmitic acid (see above) supports this conclusion. Moreover, the experiments with 20 min vasodilatation indicate that it is possible to "extract" labelled material from the intestinal cells across the "serosal" membrane during certain circumstances. This conclusion has also been confirmed in experiments using a more quantitative approach to the villous content of labelled fatty acids (Jodal and Lundgren, to be published).

Furthermore, even long chain fatty acids are normally absorbed via the blood (Saunders and Dawson 1963, Gallagher, Webb and Dawson 1965, Hyun, Vahouny and Treadwell 1967) and this route of absorption seems to be the dominant one in bile fistula rats (Saunders and Dawson 1963, Gallagher, Webb and Dawson 1965) and in puromycin treated ones (Kayden and Medick 1969). Using the everted sac method of Wilson (1956) it has, as pointed out above, been shown that free fatty

acids may to a significant extent diffuse through the entire intestinal wall (Johnston 1959 Bennett Clark 1971) Thus all these observations suggest that the serosal surface of the intestinal epithelial cell is indeed quite permeable to long chain fatty acids

It is for such reasons proposed that the uneven distribution of the labelled fatty acids between the epithelial cells and the villous stroma shown in Fig 1 2 and 3 instead reflects partly the low water solubility of palmitic acid partly a difference in binding sites for free fatty acids between the epithelial cells and the villous connective tissue core A protein binding long chain fatty acids localized in the cytosol of the intestinal mucosa has also recently been identified (Ockner *et al* 1972)

The present results favour the view that the hindrance to net blood transport of the long chain fatty acids lies *outside* the epithelial cell and is in all probability a result of the intestinal countercurrent exchanger (see introduction) The following observations from this study provide evidence for this hypothesis Firstly it was as noted above possible to extract the radioactively labelled lipid material from the epithelial cells either by placing a 7 % albumin solution in the gut lumen or by intense vasodilatation According to the countercurrent hypothesis these observations are explained by the exchanger acting as net blood transport hindrance at resting blood flows while during intense vasodilatation the exchanger becomes less efficient and then the fatty acids pass the exchanger to be transported away *via* the blood The albumin solution placed at the mucosal surface of the epithelial cell can easily extract the labelled fatty acids since no exchanger exists on this side of the cell

Secondly it seems possible to alter the concentration gradient of fatty acids along the length of the villus by making the intestine ischemic (Fig 2 and 3) This suggests that the marked gradient usually present along the villus length is caused by the countercurrent exchanged recirculating the absorbed fatty acids towards the villous tip where it is eventually built into chylomicrons During a prolonged absorption of fatty acids the tip portion is continuously being provided with absorbed fatty acids trapped in this countercurrent exchanger at the villous base and the gradient becomes more and more steep (*cf* Fig 2 and 4)

It has been generally assumed that the observed gradient of lipids along the villous length was mainly due to an enzymatic differentiation of the epithelial cells as they move from the base toward the tip of the villus (Padykula 1962) However the gradient is present already after 1 min of exposure (Fig 2) when only a small portion of the labelled fatty acids has been re-esterified Moreover a fairly even distribution of certain enzymatic activities is found in the upper 1 2—2 3 of the villi (Nordstrom Dahlqvist and Josefsson 1968)

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References

APPLETON, T. C. Resolving power, sensitivity and latent image fading of soluble compound autoradiographs. *J. Histochem. Cytochem.* 1966, *14*, 414—420.
BENNETT CLARK, S. The uptake of oleic acid by rat small intestine. A comparison of methodologies. *J. Lipid Res.* 1 1 1? 43—55.
BRINDLEY, D. N. and G. HUEBSCHER. The effect of chain length on the activation and subsequent incorporation of fatty acids into triglycerides by the small intestinal mucosa. *Biochim. Biophys. acta* 1966, *125*, 92—105.
BUSCHMAN, R. J. and A. B. TAYLOR. The effect of 0. C and 2 4 DNP on the uptake of micellar fatty acids, on the extraction of the absorbed lipid during electron microscopy processing and on the ultrastructure of everted jejunum. *J. Ultrastruct. Res.* 1971, *35*, 93—111.
CARLIER, H. Étude critique au moyen de traceurs radioactifs de la conservation des lipides des chylomicrons et de la paroi intestinale de rat en fonction de différentes techniques de préparation pour l'étude au microscope électronique. *J. Microscopie* 1968, *7*, 825—840.
CARLIER, H. Absorption en fonction du temps par un segment d'intestin de rat in situ de monopalmitine, d'acide palmitique et d'acide oléique tritié. *J. Microscopie* 1971, *12*, 193—204.
CLARK, L. M., H. C. LANZ and J. R. SENIOR. Bile salt regulation of fatty acid absorption and esterification in rat everted jejunum sacs *in vitro* and into thoracic duct lymph *in vivo*. *J. Clin. invest.* 1969, *48*, 1587—1599.
DAVSON, H. *A textbook of General Physiology*. J. & A. Churchill, London, 1970, p. 397.
DAWSON, A. M. Absorption of fats. *Brit. Med. Bull.* 1967, *23*, 247—251.
DERMER, C. B. An autoradiographic and biochemical study of oleic acid and absorption by intestinal slices including determination of lipid loss during preparation for electron microscopy. *J. Ultrastruct. Res.* 1968, *22*, 312—325.
GALLAGHER, N., J. WEBB and A. M. DAWSON. The absorption of ^{14}C oleic acid and ^{14}C triolein in bile fistula rats. *Clin. Sci.* 1965, *29*, 73—8.
GELB, A. M. and J. I. KESSLER. Effects of fatty acid structure on esterification by the small intestine *in vitro*. *Amer. J. Physiol.* 1963, *204*, 871—874.
GORDON, S. G. and F. KERN. The absorption of bile salt and fatty acid by hamster small intestine. *Biochim. Biophys. acta* 1968, *152*, 372—378.
HAMILTON, J. D. The absorption of oleic acid from emulsion and micellar solution. *Biochim. Biophys. acta* 1971, *239*, 1—8.
[illegible]ARSTROM, L., L. F. APPELGREN and S. ULLBERG. Improved method for light microscopy autoradiography with isotopes in watersoluble form. *Exp. Cell Res.* 1965, *37*, 608—613.
HOFFMAN, N. E. The relationship between uptake *in vitro* of oleic acid and micellar solubilization. *Biochim. Biophys. acta* 1970, *196*, 193—203.
HOFFMAN, N. E. and W. J. SIMMONDS. The intestinal uptake and esterification *in vitro* of fatty acid as a diffusion limited process. *Biochim. Biophys. acta* 1971, *241*, 331—333.
HYUN, S. A., G. V. VAHOUNY and C. R. TREADWELL. Portal absorption of fatty acids in lymph and portal vein cannulated rats. *Biochim. Biophys. acta* 1967, *137*, 296—305.
JERSILD, R. A. A time sequence study of fat absorption in the rat jejunum. *Amer. J. Anat.* 1966, *118*, 135—161.
JODAL, M. and O. LUNDGREN. The distribution of absorbed ^{3}H palmitic acid in the intestinal villi of the cat during various circulatory conditions. *Acta physiol. scand.* 1973, *89*, 318—326.
JOHNSTON, J. M. The absorption of fatty acids by the isolated intestine. *J. biol. Chem.* 1959, *234*, 1065—1067.
KAYDEN, H. J. and M. MEDICK. The absorption and metabolism of short and long chain fatty acids in puromycin treated rats. *Biochim. Biophys. acta* 1969, *176*, 37—43.
KOPRIWA, B. M. and C. P. LEBLOND. Improvements in the coating technique of autoradiography. *J. Histochem. Cytochem.* 1962, *10*, 269—284.
LUNDGREN, O. Studies on blood flow distribution and countercurrent exchange in the small intestine. *Acta physiol. scand.* 1967, Suppl. 303.
NORDSTROM, C., A. DAHLQVIST and L. JOSEFSSON. Quantitative determination of enzymes in different parts of the villi and crypts of rat small intestine. *J. Histochem. Cytochem.* 1968, *15*, 713—721.
OCKNER, R. K., J. A. MANNING, R. B. POPPENHAUSEN and W. K. L. HO. A binding protein for fatty acids in cytosol of intestinal mucosa, liver, myocardium and other tissues. *Science* 1972, *177*, 56—58.
PADYKULA, H. A. Recent functional interpretations of intestinal morphology. *Fed. Proc.* 1962, *21*, 873—879.
ROGERS, A. W. *Techniques of autoradiography*. Elsevier Publishing Company, 1969.
SAUNDERS, D. R. and A. M. DAWSON. The absorption of oleic acid in the bile fistula rat. *Gut* 1963, *4*, 254—260.

SAUNDERS D R J WILSON and C E RUBIN Loss of absorbed lipid during fixation and dehydration of jejunal mucosa *J Cell Biol* 1968 *37* 183—187
STRAUSS E W Morphological aspects of triglyceride absorption In *Handbook of Physiol* 1968 Section 6 (Alimentary Canal) Vol III 1377—1406
STRAUSS E and S ITO Autoradiographic and biochemical study of linolenic acid C[14] absorption by hamster intestine from mixed micelles *in vitro* *J Cell Biol* 1965 *27* 101 A
WEBB J P W J D HAMILTON and A M DAWSON A physicochemical study of fat absorption in rats limitation of methods *in vitro* *Biochim Biophys acta* 1969 *187* 42—52
WILSON T H A modified method for the study of intestinal absorption *in vitro* *J appl Physiol* 1956 *9* 137—140

Acta physiol scand 1973 89 318—326

From the Department of Physiology University of Göteborg Sweden

The Distribution of Absorbed ³H palmitic Acid in the Intestinal Villi of the Cat during Various Circulatory Conditions

By

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Abstract

JODAL M and O LUNDGREN *The distribution of absorbed ³H palmitic acid in the intestinal villi of the cat during various circulatory conditions* Acta physiol scand 1973 89 318—326

The distribution of absorbed ³H palmitic acid within the villous tissue was investigated by determining the amount of radioactivity per unit weight tissue protein in 40—50 µm thick tissue slices cut perpendicular to the long axis of the villi In most experiments a bile solution containing the tracer was incubated in the intestinal lumen for 1 min during various conditions At resting blood flow a steep gradient of radioactivity along the villous length was observed the highest amount of tracer being found at the tip This gradient was less steep if the experiments were performed during total intestinal ischemia The tissue content of ³H palmitic acid could be markedly decreased by intense vasodilatation or by placing a 7 % albumin solution on the luminal surface of the intestinal epithelial cells after removing the bile solution containing the tracer The serum albumin continuously circulating at the tissue surface of the epithelial cells was far less efficient in lowering tissue content of radioactivity The results are discussed with regard to present concepts of lipid absorption It is proposed that the results of the present investigation are explained by a "trapping" of the palmitic acid in the intestinal countercurrent exchanger efficiently hindering net blood absorption of the fatty acid

In a preceding paper (Haglund Jodal and Lundgren 1973) results based on an autoradiographic technique were presented suggesting that the partition between lymph and blood of fatty acids absorbed from the small intestine is partly explained by the countercurrent exchanger in the intestinal mucosa (see Lundgren 1967) According to this hypothesis the extremely lipid soluble long chain fatty acids are efficiently "trapped" in the countercurrent exchanger and thus correspondingly hindered from being removed by the blood stream while the less lipid soluble short chain fatty acids are less affected by the exchanger and hence mainly transported by the blood

Though autoradiography as used in the earlier study allows a good localisation of the tracer in the tissue it is at best a semiquantitative technique In order to obtain more quantitative data the present study was undertaken with a technique allowing determination of the amount of labelled lipids per weight protein in 40—50

μm thick sections of the villi consecutively cut from the tips towards the bases Such investigations were performed using radioactively labelled palmitic acid and as reported in a subsequent paper butyric acid during various circulatory conditions A preliminary report of parts of this study has been published (Haglund *et al* 1972)

Methods

A Operative and experimental procedures The experiments were performed on 20 cats anesthetized i.v. with chloralose (50 mg/kg b w) after induction with ether The animals had been deprived of food for 24 h and had no obvious signs of intestinal infection The operative procedures and the recording of the blood flow were largely similar to that described earlier (Haglund *et al* 1973) Briefly total venous outflow from 3—5 sympathetically denervated jejunal segments was recorded by a drop recorder unit operating an ordinate writer Mean arterial blood pressure was measured from the left femoral artery by a pressure transducer Each jejunal segment was cut open by thermocautery along the antimesenteric border and firmly mounted flat on a metal frame On top of the metal frame it was possible to place another frame made of plastic forming a 20 mm×40 mm bath in which the labelled solutions described below were placed in direct contact with the exposed mucosa The temperature of the solution in the bath was controlled by a thermocouple thermometer (Electrolab Copenhagen) and kept at 37 —38 C by table lamps

1 0 ml of the radioactively labelled solution (see below) was placed in the bath for a predetermined time and at various levels of blood flow in the intestinal segments Three different procedures were then used 1 After 1 or 20 min the plastic frame was removed and the labelled solution rapidly washed away with 80—100 ml of physiological saline after which the intestinal segment still mounted on its metal frame was extirpated and momentarily frozen in liquid nitrogen The flushing and freezing of a segment required less than 5 s 2 In another experimental series the segment was incubated for 1 min and rinsed with saline as described above Then 1 5 ml of a 7 % albumin solution was placed in the bath for another minute after which it was flushed away by 80—100 ml saline extirpated and frozen as described above 3 After 1 min incubation with the labelled solution the bath was rinsed with 80—100 ml saline to remove the palmitic acid Three min later the segment was extirpated and momentarily frozen in liquid nitrogen

To induce intestinal vasodilatation a constant infusion of isopropylnoradrenaline (20 μg/ml dissolved in saline) was made at a rate of 5—10 μg/min into a small branch of the superior mesenteric artery

B Isotope solution ^{3}H labelled palmitic acid was delivered dissolved in hexene benzene (50 50 Radiochemical Center Amersham stated purity > 98 %) The solvent was evaporated under N_2 and the acid was emulsified in a bile solution (gallbladder bile diluted five times with saline) so that the stock solution held a concentration of 400 μCi per ml (specific activity 500 mCi/mmol) This stock solution was diluted with bile 10 times prior to each experiment and the incubation solution thus contained 36 μCi ^{3}H palmitic acid per ml After a 2 h mixing time at 37 C during gentle shaking 1 ml of the solution was placed on the mucosal surface of each segment as described above

C Tissue sectioning A flat part from the frozen intestinal tissue was cut out in a cryostate (System Dittes Duspiva) the surface area ranging between 50—200 mm² The tissue segment was then mounted on a trimmed piece of ice on the microtome chuck with a few drops of water along the edges of the segment The margin of the mucosa 1 mm in width was removed since artifacts might have been caused by the water used for mounting of the tissue The mucosa was then sliced in 10 μm thick sections perpendicular to the long axis of the villi The part of the mounted mucosa which had not been cut within 130 μm from the first section was discarded away The amount of radioactivity per unit weight protein of intestinal tissue was then determined in 40—60 μm thick tissue slices (see below Section from different levels of the villi each 10 μm thick were placed on emulsion coated glass slides in order to make an autoradiographic control that no radioactivity had been left on the mucosal surface after flushing

D Protein and radioactivity determination The 40—60 μm thick slices were homogenized in 0 5 ml 10 % perchloric acid The precipitate was spun down and the lipids were extracted with an ether ethanol mixture (1 3 v/v) while the insoluble material was extracted in 1 5 ml 0 3 N NaOH for 30 min and then precipitated in 0 5 ml 6 N HCl The purified protein was dissolved in 0 5—2 0 ml 1 N NaOH depending upon the expected amount of protein and

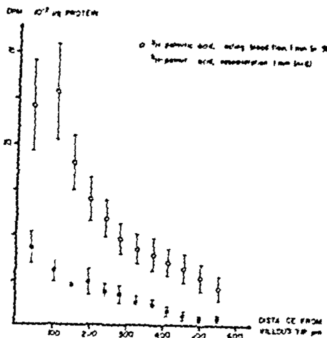


Fig. 1. The distribution of ^{3}H palmitic acid within the intestinal villous tissue expressed as DPM per mg protein. Intestinal segments in situ were exposed for 1 min to a bile solution containing the tracer during "resting" blood flow and during intense vasodilatation induced by i.a. infusion of isopropylnoradrenaline. Bars denote ± S.E.

as heated in a boiling water bath for 5 min. Total protein content was then determined according to Lowry et al. (1951) with bovine serum albumin as a standard (Sigma, Stock no. 10).

The ether-ethanol mixture containing the extracted lipids was evaporated in the counting vial at 37 °C. Liquid scintillation counting was then carried out with 10 ml of Insta-Gel (Packard) in a Packard Tri-Carb liquid scintillation counter (series 314 E). The coefficient of variation was kept below 1 % in a few samples below 2 %. Quench corrections were made by the channels ratio method or in some experiments by the internal standard method. The amount of labelled lipid per unit tissue weight was expressed as DPM/mg protein.

Results

6 different types of experiments were performed in which 1.0 ml of a bile solution containing 36 μCi (72 nmol) ^{3}H palmitic acid was placed on the exposed mucosal surface of an intestinal segment. In 2 sets of experiments the intestinal mucosa was exposed for only one minute to the labelled bile solution either during "resting" blood flow conditions (mean total venous outflow 28.0 ml/min × 100 g, range 18—34) or during maximal vasodilatation (mean flow 271 ml/min × 100 g, range 265—291) as induced by i.a. infusion of isopropylnoradrenaline. The amount of ethanol-ether extracted radioactivity per unit weight protein in transverse sections of intestinal villi is illustrated in Fig. 1 showing the considerable gradient of radioactivity along the villous length, the highest amount being found at the tip. It is also clear from Fig. 1 that the tissue content of radioactivity is considerably lower during vasodilatation than at "resting" blood flow. This observation is probably explained by a more rapid blood transport of the tracer from the villi during vasodilatation.

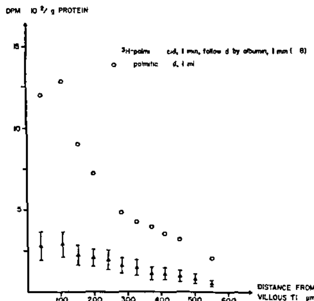


Fig 2 The distribution of ^{3}H palmitic acid within the intestinal villous tissue expressed as DPM per μg protein Triangles demonstrate the distribution pattern in experiments where intestinal segments *in situ* were first incubated for 1 min to a bile solution containing the tracer This solution was then rinsed away with a large volume of saline and the mucosa was for another minute exposed to a 7 % albumin solution As a comparison is shown the distribution pattern of ^{3}H palmitic acid after 1 min incubation during resting blood flow (Fig 1) Bars denote ± S E

From the experiments performed during resting conditions (Fig 1) it can be calculated that the average concentration of labelled palmitic acid in the upper 600 μm of the villi is 39 nmol per g villous tissue assuming the protein content of the villi to be 10 4 per cent (Jodal and Lundgren unpublished observations) About 75 per cent of the radioactivity can be removed from the tissue corresponding to about 30 nmol per g villous tissue Assuming that the radioactivity is almost exclusively located in the epithelial cells (*cf* Haglund *et al* 1973) and that the epithelial cells comprise 60 per cent of the villous volume (Jodal and Lundgren unpublished observations) the intracellular concentration of labelled palmitic acid can be calculated Such an estimation reveals that the average intracellular concentration of ^{3}H palmitic acid at the villous tips at rest (Fig 1) amounts to 192 nmol/ml tissue a figure which should be compared to its concentration in the bile solution being 72 nmol/ml

Fig 2 illustrates another way of lowering the amount of radioactivity contained in the villous tissue but during resting blood flow conditions (28 5 ml/min × 100 g range 16—40 ml) Here the mucosa was first exposed to the labelled bile solution for 1 min which was then flushed away by a large volume of saline (Methods section N 2) and exchanged for a 7 % albumin solution during another min After this procedure the amount of radioactivity remaining in the villi was only 20—30 per cent of that found without albumin exposure Evidently an albumin solution placed on the luminal side of the epithelial cells can extract a considerable fraction of their labelled lipid content

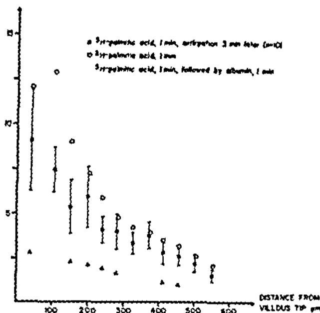


Fig 3 The distribution of ^{3}H palmitic acid within the intestinal villous tissue expressed as DPM per μg protein. Squares indicate the distribution pattern in intestinal segments which were first exposed to a ^{3}H palmitic acid containing bile solution for 1 min and extirpated 3 min later. As a comparison is shown the results obtained in two other series of experiments originally illustrated in Fig 1 and 2. Bars denote ± S.E.

In order to test whether also the albumin present in the blood passing very closely to the opposite tissue side of the epithelial cells was able to reduce their content of labelled lipids, a series of experiments was performed in which the luminal surface of the mucosa was exposed to the labelled bile solution for 1 min. The surface was then flushed with a large quantity of saline but the segment was now extirpated first after another 3 min to allow for a fatty acid elimination via

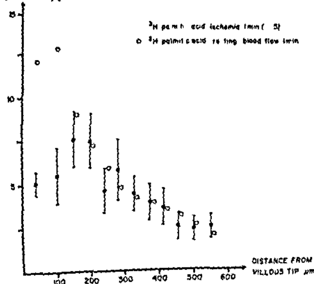


Fig. 4. The distribution of ^{3}H palmitic acid within the intestinal villous tissue expressed as DPM per μg protein. Squares indicate the distribution pattern in intestinal segments which had been exposed to a bile solution containing the tracer for 1 min during total intestinal ischemia. The results obtained at resting blood flow (Fig 1) are illustrated as a comparison. Bars denote ± S.E.

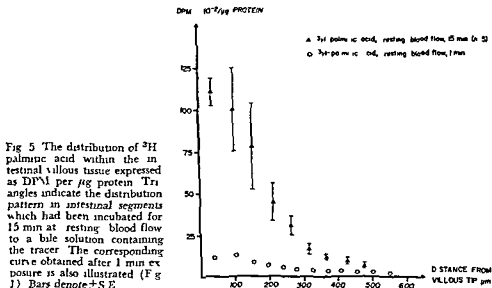


Fig 5 The distribution of ^{3}H palmitic acid within the intestinal villous tissue expressed as DPM per µg protein Triangles indicate the distribution pattern in *intestinal segments* which had been incubated for 15 min at resting blood flow to a bile solution containing the tracer The corresponding curve obtained after 1 min exposure is also illustrated (Fig 1) *Bars denote* ± S E

the albumin in the passing blood stream Mean intestinal blood flow was here 24 5 ml/min × 100 g (range 12—40) It is however quite clear from the results shown in Fig 3 that the albumin solution placed on the luminal surface of the epithelial cell is far more efficient in extracting the labelled lipids from the epithelial cells than is the albumin present in the cat s own blood circulating close to their tissue surface despite the fact that the exposure time was three times longer

In another series of experiments the luminal surface of the mucosa was exposed for 1 min to an oxygenated bile solution containing the labelled fatty acid while the blood flow to the segment was stopped by clamping the blood vessels It is evident from Fig 4 that the villous tissue content of radioactivity is similar to that found during resting blood flow except at the villous tips

Finally in one series of experiments the time of exposure to the labelled bile solution was prolonged to 15 min Mean intestinal blood flow in this series was 26 5 *ml/min × 100 g (range 20—34)* As *was also the case after* 1 *min* exposure (Fig 1) a gradient of radioactivity was apparent along the length of the villus the highest amount being present at the villous tips (Fig 5) The segment exposed for 15 min contained on an average nine times as much radioactivity per µg protein as that exposed for 1 min

Autoradiographs were made to check the amount of radioactivity remaining in the *intestinal lumen after the flushing procedures (see* Methods *section* C) In no instance was any significant graining found between villi nor was any adsorption labelled palmitic acid observed at the luminal border of the epithelial cells

Discussion

Most of the experiments of the present study were performed during 1 min exposure of the mucosa to the labelled solution. This comparatively short incubation time was chosen to assure that most of the labelled material still existed as free fatty acids (FFA), since the study of the kinetics of the FFA was the main objective of this investigation. It seems also reasonable to assume that this was the case since the authors making biochemical analyses of the tissue contents report that 50—80 per cent of the absorbed radioactively labelled long chain fatty acids exist as FFA after 1 min incubation (Hamilton 1971, Bennett Clark 1971). This is also corroborated by the present findings that 70—80 per cent of the absorbed radioactivity could be "extracted" by a 1 min exposure of the luminal surface of the epithelial cells to an albumin solution. This observation might indicate that the esterifying enzymes were exposed to too high concentrations of labelled fatty acids (cf. Hamilton 1971). However, the concentration of palmitic acid used in this study (72 nmol/ml) and the rate of its absorption (around 3.9 μmol/100 g × min) are far below the critical levels for those enzymes according to Clark, Lanz and Senior (1969), Webb, Hamilton and Dawson (1969), Hamilton (1971) and Hoffman and Simmonds (1971).

It was clearly demonstrated in this study that an albumin solution placed along the "luminal" surface of the epithelial cell was able to remove the labelled palmitic acid in the villi far more efficiently than the albumin present in the cat's own blood circulating in close contact with the tissue surface of the epithelial cells (cf. Fig. 2 and 3). This may possibly be explained by a too low transport capacity of the plasma albumin as compared to the tissue content of FFA. However, the following calculations seem to rule out such a possibility. It was calculated (see Results) that 100 g villous tissue contained an average of 3.9 μmol of the labelled acid at the end of a 1 min absorption period, i.e. about 2.75 μmol palmitic acid existed in a diffusible form (cf. Fig. 1 and 2). The villous plasma flow amounts to about 40 ml/min × 100 g villous tissue at a total intestinal blood flow of about 30 ml/min × 100 g (Biber, Lundgren and Svanvik 1973). Thus every minute 1.4 g (= 0.035 × 40) albumin was circulating along the tissue surface of the epithelial cells of an imaginary 100 g villous section. Every albumin molecule can transport at least 27 molecules FFA (Goodman 1958, Spector, John and Fletcher 1969), i.e. the maximal transport capacity for 1.4 g albumin is above 550 μmol FFA. Thus during the 3 min period of undisturbed blood flow the total transport capacity of the villous blood was more than 1500 μmol in a situation where the tissue contained only 2.75 μmol freely diffusible palmitic acid. Stated in another way, the amount of plasma albumin passing along the villous epithelium every second had a maximal transport capacity for palmitic acid that was twice as large as the *total* amount of labelled palmitic acid present in the villi.

It might be argued that the binding sites for fatty acids on the serum albumin molecules may have been already fully occupied. However, studies on the interaction of albumin with long-chain fatty acids in humans indicate that at a normal

concentration of FFA in blood only 1 out of the 27 binding sites per molecule of albumin is occupied by FFA (Goodman 1958)

The failure of the palmitic acid to be absorbed *via* the blood despite the apparently very high transport capacity of the blood albumin might be explained in case the tissue side of epithelial cells and/or the capillary walls were impermeable to the long chain fatty acids However this seems *a priori* highly unlikely since plasma membranes usually are very permeable to lipid soluble solutes Several observations made during various *in vivo* and *in vitro* conditions also support this conclusion as discussed at length in an earlier publication (Haglund *et al* 1973) The results of Fig 1 also suggest that it is possible to lower the tissue concentration of the absorbed palmitic acid by intense vasodilatation and a consequently increased blood flow apparently by a more rapid absorption *via* the blood Hence the plasma membrane was in this particular situation allowing a considerable passage of absorbed material

From the results and the discussion above one may conclude that the absorbed ^{3}H palmitic acid exists to a large extent in a diffusible form in the tissue and that the cell plasma membranes at both the luminal and the tissue sides of the epithelial cell are highly permeable to the long chain fatty acids Furthermore the blood seems to have an extremely large transport capacity for the absorbed solute and yet the palmitic acid is mainly transported away from the intestine *via* the lymph Thus the present investigation strongly favours the view that the hindrance to net blood transport of palmitic acid lies *outside* the epithelial cells a conclusion also reached by Haglund *et al* (1973)

An alternative explanation based on the countercurrent exchange mechanism in the small intestinal mucosa was proposed in a preceding paper (Haglund *et al* 1973) and presented in the introduction to this paper According to this hypothesis the long chain fatty acids are trapped in the countercurrent exchanger at resting blood flow levels and thus largely hindered from being absorbed *via* the blood (*cf* Fig 3) During intense vasodilatation on the other hand which makes the exchanger less efficient due to a high linear flow rate in the villous hairpin vascular loops blood may act as a transport vehicle al o for long chain fatty acids (*cf* Fig 1) The countercurrent exchanger does not of course hinder net diffusion across the luminal surface of the epithelial cells explaining the ability of an albumin solution placed in the gut lumen to extract the labelled fatty acid from the tissue According to the countercurrent hypothesis the concentration gradient of the absorbed fatty acid is at least partly explained by a recirculation of the absorbed material towards the villous tip During a prolonged absorption of long chain fatty acids the villous tips are continuously being provided with absorbed fatty acids and the gradient along the length of the villi becomes steeper (Fig 5) due to the re esterification of FFA to triglycerides occurring simultaneously During completed ischemia on the other hand the radioactivity gradient along the villous length is markedly reduced since the exchanger does not function at zero flow (Fig 4)

The proposed countercurrent hypothesis for the absorption of long chain fatty acids is here substantiated by experiments performed during *in vivo* conditions It is

obvious that the numerous investigations performed in vitro in the field of lipid absorption cannot possibly disclose such a mechanism. The present study underlines the necessity of studying intestinal absorption during more "physiological" conditions. This was also demonstrated for the absorption of sodium chloride where the existence of a countercurrent multiplier in the intestinal villi was proposed by Haljamäe et al. (1971).

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References

Bennett Clark, S. The uptake of oleic acid by rat small intestine. A comparison of methodologies. J. Lip. Res. 19 1 12 48—55
Biber, B., O. Lundgren and J. Svanvik. Intramural blood flow and blood volume in the small intestine of the cat as analyzed by an indicator-dilution technique. Acta physiol. scand. 1973. 87 391—403
Clark, L. M., H. C. Lanz and J. R. Senior. Bile salt regulation of fatty acid absorption and esterification in rat everted jejunum sacs in vitro and into thoracic duct lymph in vivo. J. C. r. Invest. 1969. 48 158—1599
Goodman, DeW. S. The interaction of human serum albumin with long chain fatty acid anions. J. Am. Chem. Soc. 1958. 80 3892—3898
Haglund, U., M. Jodal and O. Lundgren. The importance of the intestinal countercurrent exchanger for the absorption of fatty acids. Acta physiol. scand. 1972. 84 ? A
Haglund, U., M. Jodal and O. Lundgren. An autoradiographic study of the intestinal absorption of palmitic and oleic acid. Acta physiol. scand. 1973. 89 306—31
Haljamäe, H., M. Jodal, O. Lundgren and J. Svanvik. The distribution of Na in intestinal villi during absorption of sodium chloride. Acta physiol. scand. 1971. 83 28—
Hamilton, J. D. The absorption of oleic acid from emulsion and micellar solution. Biochim. Biophys. Acta 1971. 239 1—8
Hoffman, N. E. and W. J. Simmonds. The intestinal uptake and esterification in vitro of fatty acid as a diffusion limited process. Biochim. Biophys. Acta 1971. 41 331—333
Lowry, O. H., N. J. Rosebrough, A. L. Farr and R. J. Randall. Protein measurement with the Folin phenol reagent. J. biol. Chem. 1951. 193 265—275
Lundgren, O. Studies on blood flow distribution and countercurrent exchange in the small intestine. Acta physiol. scand. 1967. Suppl. 303
Spector, A. A., K. John and J. E. Fletcher. Binding of long-chain fatty acids to bovine serum albumin. J. Lip. Res. 1969. 10 56—6
Webb, J. P. W., J. D. Hamilton and A. M. Dawson. A physiochemical study of fat absorption in rats. Limitation of methods in vitro. Biochim. Biophys. Acta 1969. 187 42—5

Acta physiol scand 1973 89 327—333

From the Department of Physiology University of Goteborg Sweden

Studies on the in Vivo Absorption of Butyric Acid in the Small Intestine of the Cat

By

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Abstract

JODAL M and O LUNDGREN *Studies on the in vivo absorption of butyric acid in the small intestine of the cat* Acta physiol scand 1973 *89* 327—333

The effect of the intestinal countercurrent exchanger on the absorption of short chain fatty acids (SFA ^{14}C butyric and ^{3}H propion c acid) was studied *in vivo* by autoradiography and by using a technique which allowed determination of the tracer concentration along the villous length A modest concentration gradient from villous tip to villous base was found at "resting" blood flow the gradient being abolished by maximal vasodilatation or by intestinal ischemia. Furthermore the tissue content of SFA was largely uneffected by changes in intestinal blood flow The observations suggest that SFA are far less effected by the countercurrent exchanger than long chain fatty acids No evidence was provided for the existence of an active absorptive mechanism of SFA

It was recently proposed that the intestinal countercurrent exchanger described by Lundgren (1967) is one factor of importance in explaining the partition between blood and lymph of absorbed short and long chain fatty acids (Haglund Jodal and Lundgren 1973 Jodal and Lundgren 1973) This hypothesis implies that the lipid soluble long chain fatty acids become shortcircuited between the subepithelial capillaries and the central arterial vessels of the intestinal villi resulting in a hampering of their absorption *via* the blood stream This delay makes possible an almost complete synthesis into triglycerides and subsequent transport *via* the lymph The less lipid soluble short chain fatty acids on the other hand would not to the same extent be affected by the countercurrent exchanger and would therefore more readily be transported *via* the blood stream In the previous studies it was shown that two long chain fatty acids palmitic and oleic acids were affected by the countercurrent exchanger in a way predicted by the proposed hypothesis In this paper the absorption of the propionic and butyric acids was studied to explore how short chain fatty acids behave in the countercurrent exchanger

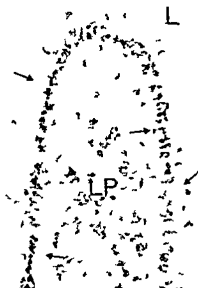


Fig 1 The distribution of ^{14}C butyric acid within the tip of an intestinal villus after 1 min incubation to a bile solution containing the tracer The lumen was carefully rinsed with 2 ml saline just prior to extirpation and freezing Arrows indicate the epithelial cell borders L = lumen LP — lamina propria Exposure time 48 days Hematoxyline eosin 380×

when the intestinal venous outflow was 33±5 ml/min×100 g (mean ±S E) The autoradiograph in Fig 1 shows the distribution of ^{14}C butyric acid in the upper half of a villus after 1 min incubation The grains are distributed diffusely within the whole villous tissue with the same density in both the epithelial cells and the lamina propria The same distribution pattern was seen when the incubation period was prolonged to 5 or 15 min or when ^{3}H propionic acid was used as tracer These results differ markedly from those earlier reported for ^{3}H palmitic and ^{3}H-oleic acid (Haglund *et al* 1973) where the grains were found predominantly in the epithelial cells

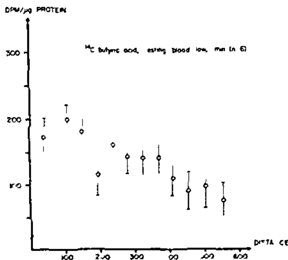


Fig 2 The distribution of C butyric acid within the intestinal villous tissu expressed as DPM per µg protein The mucosa of the intestinal segments in were exposed for 1 min to a bile solution containing the tracer at resting blood flow Bar denot ±S E

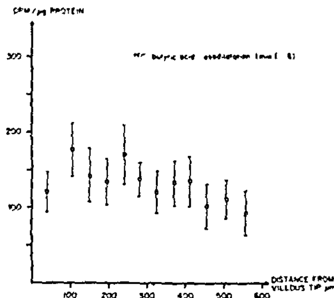


Fig 3 The distribution of ¹⁴C butyric acid w... n the in testinal v.lous tis e expressed as DPM per μg protein. The mucosa of intestinal segments in situ were exposed for 1 min to a bile solution contain... the tracer during intense vaso dilatation Bars deno e ± S E

In order to study the distribution of butyric acid in a more quantitative ma... throughout the entire villi the amount of radioactivity per unit weight protein al... the long axis of the villi was determined as described in Methods section D a... different levels of blood flow Fig 2 illustrates the villous distribution of radioactivity during resting blood flow (32 ± 8 ml/min$\times$100 g n = 6) after 1 min intralum... incubation of ¹⁴C butyric acid in bile The amount of radioactivity per μg protein showed a modest gradient from tip to base During intense vasodilatation (... 34 ml/min$\times$100 g n = 6) a more even distribution pattern was seen as illustrated in Fig 3 The average value of DPM/μg protein in the villus was the same d...

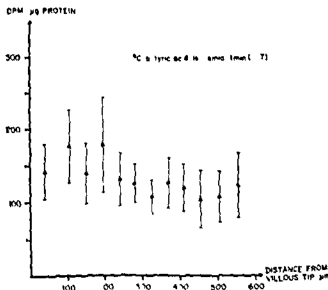


Fig 4 Distribution of ¹⁴C butyric acid within the in testinal villous tissue expressed as DPM per μg protein. The mucosa of intestinal segments in situ were exposed for 1 min to a bile solution contain... the tracer during total ... mia Bars denote ± S E

resting blood flow (114 ± 26 DPM/μg protein) as during maximal vasodilatation (115 ± 24 DPM/μg protein) These values correspond to a tissue concentration of 0 32 μmol/ml tissue assuming the villous protein concentration to be 10 4 g/100 g tissue (Jodal and Lundgren unpublished observations) This indicates that an eightfold increase in total intestinal blood flow did not result in any significant decrease of the ^{14}C butyric acid concentration in the villous tissue

These observations were also corroborated by autoradiographic experiments in which intestinal blood flow in 3 cats was increased from resting levels to 155 ml/min × 100 g (range 100—260 ml/min × 100 g) during a 5 min incubation period with ^{14}C butyric acid in a bile solution The distribution of the grains in the auto radiographs were similar to that seen in Fig 1 *i e* the same grain density was observed in the epithelial cells as in the lamina propria Moreover no gradient along the villous length was observed

Incubation of an oxygenated ^{14}C butyric acid bile solution for 1 min during complete obstruction of intestinal blood flow achieved by clamping the mesenteric vessels was performed in 7 expts (Fig 4) The radioactivity was also now fairly uniformly distributed being 137 ± 38 DPM/μg protein and corresponding to a tissue concentration of 0 38 μmol/ml tissue

The water-oil partition coefficient at pH 7 0 was estimated to be 497 ± 8 (mean ± S E n = 4) for ^{14}C butyric acid and 0 43 ± 0 01 (n = 6) for ^{14}C-decanoic acid Thus at the pH of the bile solution used in this study butyric acid was almost entirely found in the water phase being more than thousand times more watersoluble than decanoic acid

Discussion

According to the hypothesis outlined in the introduction the countercurrent exchanger in the intestinal villi would act as a hindrance to net blood absorption of the lipid soluble long chain fatty acids while the short and medium chain fatty acids being far less lipid soluble would not be affected to the same extent by the exchanger In two earlier studies (Haglund *et al* 1973 Jodal and Lundgren 1973) this hypothesis was tested as regards the absorption of palmitic and oleic acid and several of these observations were consistent with the countercurrent hypothesis First a steeply decreasing gradient of radioactivity from the villous tip towards the base was evident already after 1 min exposure evidently to a large extent due to an uneven distribution of the nonesterfied palmitic acid According to the counter current hypothesis this was caused by an extravascular crossdiffusion of the absorbed fatty acids from the subepithelial capillary network to the central vessel with a subsequent blood transport towards the villous tip Second the concentration gradient along the villous length of the absorbed palmitic acid was markedly decreased when blood flow was stopped so that no countercurrent exchange concentration could occur towards the villous tip Third the tissue concentration of palmitic acid was markedly decreased by increases of intestinal blood flow which would lower the efficiency of the exchanger in hindering net blood absorption by reducing the time

available for cross diffusion between the descending and ascending vascular limbs.

The results presented in the present investigation where the almost exclusively water soluble butyric acid was studied are entirely different from those just described for palmitic acid. Thus only a very modest radioactivity gradient was observed at "resting" blood flow. Furthermore neither ischemia nor intense vasodilatation caused any marked changes in the distribution pattern or tissue content of the tracer. These observations are in full agreement with the hypothesis that butyric acid is far less affected by the countercurrent exchanger. The low lipid solubility of the ionized form of butyric acid, being of the same order of magnitude as e.g. urea, apparently restricts its passage across the capillary wall to such an extent that not even a marked vasodilatation was able to decrease the tracer tissue content in the experimental situation of the present study. This conclusion is also corroborated by the observation that the rate of blood absorption of ^{14}C-butyric acid is only slightly augmented when intestinal blood flow is increased from a "resting" level to maximal vasodilatation (Jodal and Lundgren, unpublished observations). Thus in this respect butyric acid seems to behave like urea, the absorption rate of which seems to be largely unaffected by changes in blood flow (Winne and Remischowsky 1971). Urea has also been shown to be comparatively little affected by the mucosal countercurrent exchanger (Kampp, Lundgren and Sjöstrand 1968).

It should be underlined that the cell permeability of weak acids such as butyric acid or propionic acid is markedly pH-dependent (cf. Hogben et al. 1959), the nonionized form being lipid soluble and easily diffusible across plasma membranes while the ionized form, as pointed out above, is mainly water soluble. In this study the luminal pH was kept around 7.0 compared to the extracellular tissue pH being normally around 7.4. As pK for all fatty acids is around 4.8, only a very small fraction of butyric acid exists in a non ionized form at a pH of 7—7.4. However, intracellular pH is not exactly known but assumed to be somewhat less than 7.0 (Caldwell 1956) which makes it difficult to predict the rate of passage of butyric acid in and out of the intestinal epithelial cell. Further, it has been proposed by Hogben et al. (1959) that weak acids pass through the plasma membrane exclusively in their non-ionized form and that the luminal pH close to the absorption surface of the epithelial cells is low, being around 5.3. Butyric and propionic acid may therefore pass more easily across the plasma membranes of the epithelial cells than the capillary wall due to regional differences in pH.

With increasing chain length the fatty acids become more and more lipid soluble. Hence their plasma membrane and capillary permeability increases (Oldendorf, personal communication). However, the "trapping" effect of the intestinal countercurrent exchanger with respect to the absorbed acids becomes more and more effective the higher their lipid solubility. The net rate of their absorption via the blood stream will therefore be determined by these two opposing factors. The situation is further complicated by the preference of the esterifying enzymes for certain fatty acids in the apical parts of the epithelial cells. From results reported in the literature it seems as if the moderate degree of lipid solubility as exhibited by the

medium chain fatty acids represents the most advantageous situation since these acids are most rapidly absorbed (Dawson Holdsworth and Webb 1964 Hyun Vahouny and Treadwell 1967 Gallagher and Playoust 1969 Block Haberich and Lorentz Meyer 1972)

An active transport mechanism across the intestinal epithelium has been proposed to exist for short chain fatty acids (Smyth and Taylor 1958 Barry and Smyth 1960 Barry Jack on and Smyth 1966) Although the present study was not intended to investigate this particular question one may expect such a mechanism to create clearcut concentration differences of the absorbed short chain fatty acids within the villous tisues particularly at epithelial cell boundaries However such an uneven distribution was not apparent in the autoradiographs Furthermore the tissue concentration of labelled butyric acid as calculated in this study was always below that of the incubated solution except during resting blood flow when the tip portion of the villus seemed to reach about the same concentration of tracer as in the bile olution Thus the present study did not provide any evidence for the existence of an active absorption mechanism of short chain fatty acids

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AB Vitrum generously supplied us with heparin Thanks are al o due to AB Pharmacia for its supply of Rheomacrodex® and Macrodex®

References

APPLETON T C Resolving power sensitivity and late it image fading of soluble compound autoradiographs *J Histochem Cytochem* 1966 *14* 414—420
BARRY R J C M J JACKSON a id D H SMYTH Transfer of propionate by rat small intestine *in t tro J Physiol* (Lond) 1966 *182* 150—163
BARRY R J C and D H SMYTH Transfer of short chain fatty acids by the intestine *J Phys l* 1960 *152* 48—66
BLOCH R F J HABERICH and H LORENTZ MEYER Untersuchungen zur Transport kinetik m ttelkettiger Fettsauren am Dunndarm *Pflugers Arch ge Physiol* 1972 *335* 198—212
CALDWELL P C Intracellular pH *Int Re Cytol* 1956 *5* 229—277
DAWSON A M C D HOLDSWORTH and J WEBB Absorption of short chain fatty acids in man *Proc Soc xp Biol* (N Y) 1964 *117* 97—100
GALLAGHER N D and M R PLAYOUST Absorption of saturated and unsaturated fatty acids by rat jejunum and ileum *Gastroenterology* 1969 *57* 9—18
HAGLUND U M JODAL and O LUNDGREN An autoradiographic study of the intestinal absorption of palmitic and oleic acid *Acta physiol scand* 1973 *89* 306—317
HOGBEN C A M D J TOCCO B B BRODIE and L S SCHANKER On the mechanism of intestinal absorption of drugs *J Pharmac exp Ther* 1959 *125* 274—282
HYUN S A G V VAHOUNY and C R TREADWELL Po tal al orpt on of fatty acids in lymph and portal ein cannulated rats *Biochim Biophys acta* 1967 *137* 296—305
JODAL M and O LUNDGREN The distribution of absorbed ^{3}H palmitic acid in the intestinal villi of the cat during various circulatory cond tio s *A ta phys l s a d* 1973 *89* 318—326
KAMPP M O LUNDGREN and J SJOSTRAND The di t ib ıt on of int a a cula ly administered l pid soluble and lipid insoluble su bstance in the mucosa nd th subm ucosa of the small intestine of the cat *Acta phys l ca d* 1 t 8 7 469—480
LUNDGREN O Studies on blood flow d trib t on nd cour terc ent exchange in th m ll intestine *Acta physiol sca d* 1967 S ppl 303
SMYTH D H and C B TAYLOR Intest nal transf of hort cha n fatty acids in vitro *J Physiol* (Lond) 1958 *141* 73—80
WINNE D and J REMISCHOWSKY D r Einflu s d r Durchblutung auf die Resorption von Harnstoff Meth nol und Ethanol s d m Jejunum der Ratt *Nau yn-Sm ed be g A ch Path Pharmak* 1971 *268* 39 —416

Acta physiol scand 1973 89 334—341
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On the Possible Role of Bradykinin in Functional Hyperemia of Cat's Stomach

By

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Abstract

Fasth S and J Martinson *On the possible role of bradykinin in functional hyperemia of cat's stomach* Acta physiol scand 1973 89 334—341

The reactions of the consecutive vascular sections of the stomach were studied with a plethysmographic technique at "rest" during graded and maximal vasodilatation induced by isoprenaline and during infusion of bradykinin supposed to be involved in functional hyperemia evoked by vagal nerve stimulation It is shown that the stomach has a great capacity to maintain a rather constant area for filtration even during large variations in peripheral resistance Bradykinin evoked an atropine resistant vasodilatation This is similar to the effect of vagal stimulation In contrast bradykinin did not increase capillary permeability which is one effect of recognized for vagal stimulation It is concluded that this plasmakinin may not play a role of functional hyperemia in the stomach since the whole pattern of vascular effects known for vagal stimulation is not reproduced

The kinins are a group of biologically highly active polypeptides, acting on the smooth musculature of the gastrointestinal tract evoking vasodilatation in most vascular beds and increasing vascular permeability (for review see Erdos 1966) They are probably involved in physiological as well as in pathophysiological events (for review see Kellermeyer and Graham 1968)

During recent years the evidence for the kinins as mediator of functional hyperemia in salivary glands (Gautvik 1970) and pancreas (Hilton and Jones 1968) has accumulated A similar functional hyperemia may be evoked in the stomach by stimulation of high threshold vagal fibers and it has been proposed that this may be the result of a specific plasmakinin mechanism (Martinson 1965) There are also some observations indicating that stimulation of the vagal nerves increases the permeability of the capillaries even after total gastric blood flow has returned to the control level after the stimulation (Jansson Lundgren and Martinson 1970) The effect of vagal stimulation on the consecutive vascular sections of the stomach therefore seems to be mimicked by that of bradykinin on other vascular beds e g that of the colon (Fasth and Hulten 1973)

The aim of the present investigation was to study the qualitative and quantitative effects on the series coupled vascular sections of the stomach and to explore whether bradykinin might possibly affect blood flow resistance and capillary permeability in the same direction and to a similar extent as vagal stimulation This would lend support at least indirectly to the hypothesis that the kinins might be mediators in the vagal nerve response

Material and methods

Operative procedures Experiments were performed on 25 cats weighing between 1 9 and 5 5 kg The animals fasted for 24 h were anesthetized i v with chloralose after induction with ether A tracheal cannula was inserted to maintain the air passage A femoral artery was connected to a mercury manometer for continuous recording of arterial blood pressure The animals were heparinized (3 mg/kg) and atropinized (1 mg/kg)

The abdominal cavity was opened in the midline and the spleen the greater omentum, the pancreas the small intestine and colon were extirpated The oesophagus was sectioned just beneath the diaphragm The common bile duct and the hepatic artery were divided

Blood flow and tissue volume recordings To measure the total gastric blood flow a wide bore polyethylene tube was inserted into the portal vein which after the above mentioned operative procedure drained only the stomach and surrounding lymphatic and adipose tissue The venous outflow was then diverted to a drop chamber with a photoelectric dropcounter operating an ordinate writer The blood was then returned to the animal via the external jugular veins In order to place the stomach in a plethysmograph the celiac artery had to be di ided and con nected in most experiments to one carotid artery via siliconized tubes of wide dimensions The whole preparation could then be placed in the perspex plethysmograph which was connected to a welltrimmed piston recorder to record volume changes. The plethysmograph was filled with isotonic saline kept at 38 C A rubber tubing inserted into the pylorus was fitted through an opening in the plethysmograph for draining the stomach (Fig 1)

Determination of the capillary filt ation coeffici nt After enclos ng the stomach in the plethysmograph it was possible to adjust the venous outflow pressure so that no changes in volume appeared indicating that net transcapillary fluid movement was zero This isovolumetric state could then be deliberately changed by temporarily increasing the venous outflow pressure by a known amount (10 cm H_2O) This procedure evoked a characteristic volume increase in two phases the first one caused by distension of mainly the thinwalled veins and the second one caused by the raised mean capillary hydrostatic pressure (Mellander 1960) The capillary filtration coefficient as a measure of capillary exchange area and capillary permeab lity ex pressed in ml of fluid filtered across the capillary wall per min in 100 g (ml/min × mmHg × 100 g tissue) was calculated assuming a mean hydrostatic cap llary pressure of 15 mmHg at a venous outflow pressure close to zero This calculation is based on the extent to which the increase of venous pressure is propagated to the capillary bed i e on the relation between pre and postcapillary resistances of the vascular bed When studying the effect of a vasoactive drug on that very vascular bed the fraction of the applied venous pressure which is transmitted to the capillaries might be changed This would introduce an error in the calculation of CFC How ever if the iso olumetric equilibrium is not disturbed by the drug under study it may be as sumed that the pre to postcap llary resistance ratio is un hanged Furth rmo by first bring ing the vascular bed to maximal dilatation by an unspecif c vasodilato th effect of another vasod latory drug on the pre to postcapillary resistances an be avoided the eby permitting selective stud es of the effects on the porosity of the e hange essels

Peripheral resistance was calculated as the ratio of mean arterial pressu to blood flow (ml/min × 100 g tis ue) and expressed in peripheral resistance un ts (1 PRU$_{100}$ = 1 mmHg/ ml/min × 100 g)

Adm n st ation of drugs Synthetic bradyk nin (kindly supported by Bofors Pharma) was administered as close intraarterial infus on r tes arying betwe n 0 04—0 48 ml/m n. The sub stance was dissol ed n 0 9 % saline n concentration between 0 1 and 100 μg/ml The con centration in the arterial whole blood was calculated To nd ce graded and maximal vaso dilatat on intraarteri l infusion of isoprenaline sulphate wa performed in some experi After the experiments the weights of the stoma h and adjacent mesenteric t ssue w mined.

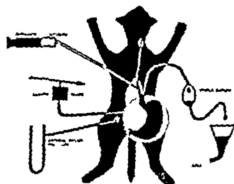


Fig. 1. Schematic illustration of the technique used for studying the reactions within the consecutive vascular sections of the stomach.

Results

I. *Basal conditions and infusion of isoprenaline.* Resting blood flow in the acutely denervated stomach varied between 9 and 44 ml/min×100 g tissue (mean 19.0± 8.3, S.D., n = 73) while regional vascular resistance ranged between 13.5 and 2.7 PRU_{100} (mean 7.0+2.88 S.D.). Intraarterial infusions of supramaximal doses of isoprenaline increased blood flow to approximately 70 ml/min×100 g tissue and reduced resistance to at most 1.0 PRU_{100}. The gastric capillary filtration coefficient (CFC), as a measure of the capillary surface available for filtration, was at resting blood flow approximately 0.04 ml/min×mmHg×100 g tissue and increased to about 0.08 when the vascular bed was maximally dilated. The correlation between the peripheral resistance and CFC at different levels of vascular tone is shown in Fig. 5.

II. *The effect of intraarterially administered bradykinin*

A. *Resistance vessels.* Intraarterial infusion of bradykinin invariably caused an increase of the blood flow provided that the concentration of the substance exceeded 0.001 μg/ml perfusing blood (Fig. 2). The increase in blood flow occurred immediately and was well maintained during the infusion. On cessation of the infusion there was an immediate return to control level (Fig. 3). The blood flow responses were dose dependent with a maximal vasodilatation amounting to some 60 ml/min× 100 g tissue at a blood concentration of about 1 μg/ml blood. The systemic arterial blood pressure was never affected. Tachyphylaxis did not appear.

B. *Tissue volume.* When bradykinin was administered in doses that did not evoke vasodilatation there was no effect on the capacitance vessels. On increasing the doses, however, there was concomitantly with the blood flow increase a rapid initial increase of tissue volume reflecting an increase of the regional blood volume. On cessation of the infusion the tissue volume decreased rapidly and simultaneously with the decrease of the blood flow. After maximal vasodilatation induced by means of supramaximal doses of isoprenaline superimposed infusion of even large doses of bradykinin had no effects on the capacitance vessels (Fig. 4). The initial rapid increase of the tissue volume (Fig. 3) was followed by a slower increase that gradu-

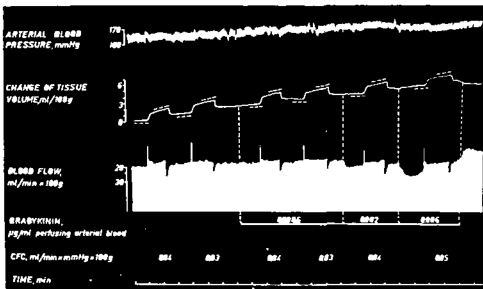


Fig 2 Cat 2 1 kg Effects of i a. bradykinin infusion on the consecutive vascular sections of the stomach Note the slight increase of the blood flow at 0 002 µg/ml blood and a further increase at 0 006 µg/ml Note also the absence of effects on the CFC apart from the slight increase during the highest blood flow

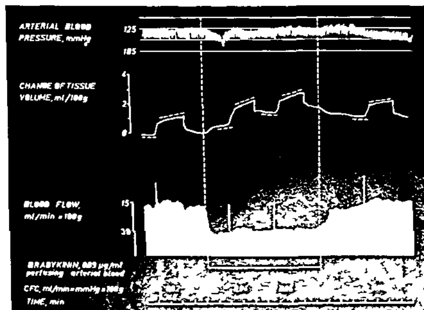


Fig 3 Cat 3 2 kg Effects of i a bradykinin infusion on the consecutive vascular sections of the stomach Note the rapid increase of blood flow an initial increase of tissue volume a new "isovolumetric" state during infusion and a tendency to decrease in tissue volume after cessation of the infusion. Note also the rapid decrease of CFC to the "resting" value

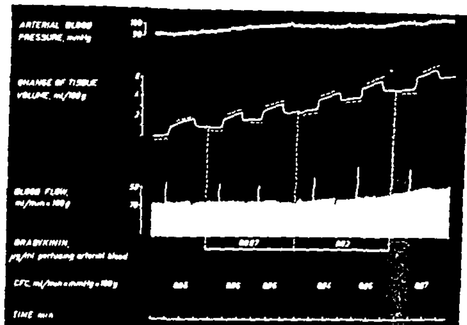


Fig 4 Cat 2 8 kg The effect of intraarterial bradykinin infusion on the consecutive vascular sections of the stomach during maximal vasodilatation as induced by supramaximal doses of isoprenaline Note the absence of effects on the CFC during bradykinin infusion in doses known to elicit vasodilatation

ally subsided however In some minutes there was a new *isovolumetric* state and after cessation of the infusion the tissue volume tended to decrease somewhat However at maximal and supramaximal doses of bradykinin as regards blood flow increase there was sometimes a pronounced continuous increase of the tissue volume amounting to at most 3—4 ml/min × 100 g tissue At termination of the infusion there was simultaneously with the rapid decrease of blood flow a decrease of the volume corresponding to the diminished blood volume After this decrease the tissue volume did not diminish further

Subthreshold doses as regards the effect on the resistance and capacitance vessels caused no effect on the exchange vessels (Fig 2) An increase of the blood flow was followed by an increase of the capillary filtration coefficient (Fig 3) but as shown in Fig 5 the CFC figures during bradykinin infusions causing an increase of the blood flow did not significantly differ from the figures at the same blood flow during resting conditions After cessation of the infusion the CFC values rapidly returned to control levels To definitely exclude an effect on the capillary permeability the vascular bed was brought to maximal vasodilatation by means of isoprenaline exposing a maximal surface available for filtration As is outlined in Fig 4 and 5 superimposed administration of bradykinin in doses which regularly would induce a vasodilatation caused no further increase of the CFC

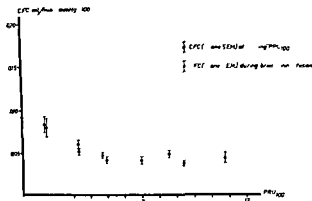


Fig 5 Cumulated and grouped data (no 171) on the correlation between gastric PRU and CFC at different levels of vascular tone at rest and during bradykinin infusion The values at approximately 1 PRU_{100} (to the left) was recorded during i.a. infusions of isoprenaline As is shown there was no further increase of CFC caused by the bradykinin infusion The blood concentration of bradykinin exceeded at all infusions the levels necessary to cause a decrease of PRU

Discussion

In the present series of experiments the resting gastric blood flow was calculated to about 20 ml/min × 100 g tissue which correspond fairly well to reported figures on the cat stomach in a less extensive preparation (Martinson 1965) The capillary filtration coefficient CFC at different levels of vascular tone appears not to have been studied previously In the pre ent investigation CFC was fairly constant during resting conditions and calculated to about 0 04 ml/min × mmHg × 100 g tissue and could be increased further to about 0 08 during maximal va odilatation as induced by supramaximal doses of isoprenaline The basal values are almost of the same order of magnitude as tho e reported for the colon during resting conditions after acute decentralization (Hulten 1969) The CFC values however at maximal vasodilatation are considerably lower in the stomach than in colon (Hulten 1969) When compared with the CFC values of the small intestine those of the stomach are considerably lower both at rest and during maximal vasodilatation at least when compared with the values of CFC reported by Folkow *et al* (1963) The blood flow interpreted as maximal was only about 70 ml/min × 100 g in the stomach as compared with about 200 or more in the small intestine and colon The discrepancy may at least in part be due to a comparatively greater amount of adipo e tissue in the stomach preparation This tissue amounted to roughly 50 % of the total preparation

The capacity of the consecutive vascular sections of the canine subcutaneous adipose tissue has recently been studied by a similar technique as in the present investigation (Fredholm Öberg and Rosell 1970) The resting blood flow averaged 9 5 ml/min × 100 g and the maximal blood flow induced by isoprenaline approximately 25 ml/min × 100 g The corresponding CFC figures were about 0 02 and 0 03 ml/min × mmHg × 100 g respectively Provided similar properties of the adipose tissue adjacent to the stomach of the cat the gastric blood flow and CFC would *e g* during maximal vasodilatation in the present study have reached figures of some 110 ml/min × 100 g and 0 12 respectively Fredholm *et al* also showed that bradykinin increased the porosity of the capillaries in the adipose tissue which means

the gastric CFC figures during infusion of bradykinin would if anything be even lower than those recorded

Despite a rather great range of variations in peripheral resistance there were only small variations in the capillary filtration coefficient It thus seems that the stomach has a great capacity to maintain a rather constant capillary area available for filtration presumably by variations in the tonus of the precapillary sphincters

The effect of bradykinin on the resistance vessels of the stomach was quite similar to that reported in the colon (Fasth and Hulten 1973) Vasodilatation occurred at a blood concentration of bradykinin exceeding 0 001 μg/ml This is far below the dose reported by Jacobson (1964) in studies of the effect of bradykinin on the canine gastric blood flow during constant flow perfusion Jacobson also observed a secretory effect of bradykinin He suggested that the secretory effect was a result of injury and transudation rather than a primary effect on the parietal cells and that the most likely reason for the appearent vascular insensitivity was a technical one In the present investigation however, bradykinin exhibited an effect on the consecutive sections of the vascular bed similar to other purely vasodilating drugs as the increase of the capillary filtration coefficient seemed to be linked to an increase of the surface available for filtration This CFC increase rapidly returned to control levels on cessation of the infusion A true damage of the cells should probably have been revealed by a progressive tissue swelling and an increase of the capillary filtration coefficient due to an increased permeability

As mentioned above it has been proposed that a plasmakinin mechanism might be involved *in the functional vasodilatation induced by vagal nerve stimulation* It has been shown that glandular cell homogenate of the stomach contains the kinin releasing enzyme kallikrein (Amundsen and Nustad 1966) However to test the hypothesis that the kinins mediate functional vasodilatation induced by vagal stimulation it is also necessary to show that the kinins may mimick the effect of vagal stimulation at least as regards the effects on the vascular bed The present investigation shows that bradykinin causes a vasodilatation which is resistant to atropine In contrast the vagal stimulation effect on the exchange vessels as reported (Jansson *et al* 1970) could not be reproduced as there was no increase of the capillary permeability

Thus the whole pattern of the vascular effects caused by vagal stimulation is not reproduced by bradykinin and therefore it is unlikely that this very plasma kinin plays the role of mediator of functional vasodilatation in the stomach It is however, a rather puzzling finding since bradykinin has been shown to increase capillary permeability even at lower doses than those necessary to decrease blood flow resistance considerably in the colon (Fasth and Hulten 1973) and in most other tissues including adipose tissue (Fredholm *et al* 1970) On very high doses there was sometimes however a considerable tissue swelling of the same magnitude as may appear in the intestine It is therefore also tempting to speculate in specific resistive properties of the capillary bed of the stomach at least when the role of bradykinin in pathophysiological events is to be discussed

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References

AMUNDSEN E and K NUSTAD Kinin forming and destroying activities of cell homogenates *J Physiol* (Lond) 1965 *179* 479—488

ERDOS E G Hypotensive peptides Bradykinin kallidin and eledoisin *Advanc Pharmacol* 1966 *4* 1—89

FASTH S and L HULTEN Neurohumoral regulation of motility and blood flow in the colon *Experientia* (Basel) 1973 *29* 3 296—297

FOLKOW B O LUNDGREN and I WALLENTIN Studies on the relationship between flow resistance capillary filtration coefficient and regional blood volume in the intestine of the cat *Acta physiol scand* 1963 *57* 270—283

FREDHOLM B B B ÖBERG and S ROSELL Effects of vasoactive drugs on circulation in canine subcutaneous adipose tissue *Acta physiol scand* 1970 *79* 564—574

GAUTVIK K Studies on kinin formation in functional vasodilatation of the submandibular salivary gland in cats *Acta physiol scand* 1970 *79* 174—187

HILTON S M and M JONES The role of plasma kinin in functional vasodilatation in the pancreas *J Physiol* (Lond.) 1968 *195* 521—533

HULTEN L Extrinsic nervous control of colonic motility and blood flow *Acta physiol scand* 1969 Suppl 335

JACOBSON E D Hemodynamic effects of bradykinin and gastrin in the stomach *Amer Heart J* 1964 *68* 214—219

JANSSON G O LUNDGREN and J MARTINSON Neurohormonal control of gastric blood flow *Gastroenterology* 1970 *53* 425—429

KELLERMEYER R W and R GRAHAM Kinins-possible physiologic and pathologic roles in man *New Engl J Med* 1968 *279* 754—759 802—807 859—866

MARTINSON J The effect of graded vagal stimulation on gastric motility secretion and blood flow in the cat *Acta physiol scand* 1965 *64* 301—309

MELLANDER S Comparative studies on the adrenergic neuro-hormonal control of resistance and capacitance blood vessels in the cat *Acta physiol scand* 1960 Suppl 176

Acta physiol scand 1973 89 342—351

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Effect of Body Temperature on Steady State Ventilation and Metabolism in Exercise

By

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Abstract

PETERSEN E S and H VEJBY CHRISTENSEN *Effect of body temperature on steady state ventilation and metabolism in exercise* Acta physiol scand 1973 89 342—351

Four healthy subjects were studied at rest and during steady state of work at normal room and body temperature and at elevated body temperature (38.5 °C) in a climatic chamber. Ventilation, oxygen uptake, carbon dioxide elimination, heart rate and blood lactate and pyruvate concentrations were measured. At equal work loads ventilation was not different, although respiratory rate was consistently higher and tidal volume lower at elevated temperature. Oxygen uptake was lower and the ventilatory equivalent therefore higher in hyperthermia than in normothermia. Blood lactate concentration was higher both at rest and at all work loads indicating an increased anaerobic energy yield in hyperthermia. The study indicates the existence of a temperature threshold near 38 °C above which a relative hyperventilation is seen. The observed hyperventilation is hardly caused by changes in the $[H^+]$ stimulus and it is suggested that hyperthermia *per se* or through interaction with other stimuli might constitute additional ventilatory drive.

Temperature has been suggested as a ventilatory stimulus both at rest and during exercise. Temperature might exert an effect directly on thermosensitive receptors in the skin, muscle (Morgan *et al* 1955), arterial chemoreceptors (Bernthal and Weeks 1939, Witzleb 1952) or within the brain (Chai, Mu and Brobeck 1965, Tabatabai 1972) or indirectly by increasing the sensitivity of proprioceptors or by increasing the response of chemoreceptors and/or central respiratory neurones to "normal" stimuli (Cunningham and O'Riordan 1957). An effect of temperature might also be unspecific, related to the concomitant decreased efficiency of CO_2 buffering in the body fluids or to an increase in $[H^+]$ (Alexander, Workman and Lambertsen 1962).

While at rest a thermal effect on breathing is well established (Haldane 1905, Bazett and Haldane 1921, Landis *et al* 1926, Cunningham and O'Riordan 1957) in exercise there is no conclusive evidence of neither separate nor amplifying thermal influences (Cotes 1955, Dejours *et al* 1958, Whipp and Wasserman 1970).

The possible interaction of neurogenic reflexes and hyperthermia on the ventilatory control at transitions from rest to work and from work to rest has been discussed in

TABLE I Physical data of the subjects Body w ght at the start and end of the experiment and, water intake in the climatic chamber

Subject	Sex	Age	Height (cm)	Body weight (kg) Start	End	Water intake (l)
LJ	F	19	169	61 970	61 470	1 310
ILK	F	22	178	71 730	70 740	1 350
PB	M	21	183	73 330	71 400	0 900
NHB	M	23	190	78 140	77 570	0 600

another report from this laboratory (Vejby Christensen and Strange Petersen 1973 a) The total output from the respiratory center as estimated by changes in expired minute ventilation was in that study not found significantly altered by increasing body temperature

In a further attempt to evaluate the possible significance of temperaturee as a respiratory drive in man during exercise the present study was planned to investigate steady state ventilation and its two components rate and depth in relation to O_2 uptake and CO_2 elimination at various loads at normal and at elevated body temperature

Methods

4 healthy subjects entered the study (Table I) They were all relatively fit but none of them were acclimatized to work in warm and humid surroundings.

The experiments on each subject were performed on 3 separate days For each subject two experimental sessions were carried out at normal body temperature in a climatically comfortable laboratory (dry bulb temperature about 24 C relative humidity about 50 %) and one in an environmental chamber (Andersen and Lundq ist 1970) with dry bulb temperature about 44 C and high relative humidity A rise in body temperature to about 38 5 C (38 4—38 7 C) was induced by placing the subjects in thi chamber for a warming up period lasting abo it one hour with relative humidity as high as 75 % By changing relati e humid ty and sometimes using a revolving fan to ncrease a r flow around the subject, rectal temperature was held within narrow limits during the experiments.

During each of the two experimental sessions at normal temperature the subjects were stud ed at rest and at two loads of exerc e on a bicycle ergometer (Elema Schonander) In the climatic chamber the subjects were studied at rest and—with one exception (*subj* PB)—at 3 loads Between each work load the subjects rested in a chair for at least 30 min The measu ements were for 3 of the 4 subjects carried out in the course of e periments described elsewhe e (Vejby Chri t nsen and Strange Petersen 1973 a)

The subjects breathed room air th ough a low resi tance mall dead space (25 ml) val e In each steady state (d fined as a state he e heart rate on_tant which as u ually the case after 3 occasionally 4 m nutes of work) expired gas as ollected n a Douglas bag in a 3 minute period and amples were tak n fo d termir at on of O and CO_2 content using the micro-Scholander method (Scholander 1947) The volu of the bag was measured with a dry gas-meter placed in the lunatic chamber This allowed n asurem ents of exp red minute olume ($\dot{V}_E$ l/min BTPS) and calculations of ox gen uptake ($\dot{V}_{O_2}$ l/m n STPD ar d carbon diox de elimination ($\dot{V}_{CO_2}$ l/min STPD) R p rators f q enc as obtained f om the tracing of CO_2 concentrat ons at the mouth The end t dal CO_2 ten on was re c ded w th an infrared CO_2 meter (Beckman) but becau e of la g o- and rangedrift bet een the cal l rations during the experiments at elevated temperatu th alue w ll ot be repo t d

Heart rate was obtained e th f om continuo s ECG reco d ng o wh n elect ode sk n contact deter orated during xtrei w ating I n ar mou ted pul meter (SAN FI Instruments) Arte t lized ar illary blood was sampled f om h a lobe t re t and in the steady stat of ea h wo k load Blood d pped freel into tubes w th s d perchlo c acid after thorough m xing the t bes were immediately placed at 4 C La t te and pyruva e co tions were measur d w th the enzymatic fluorimet c method des bed b O n (

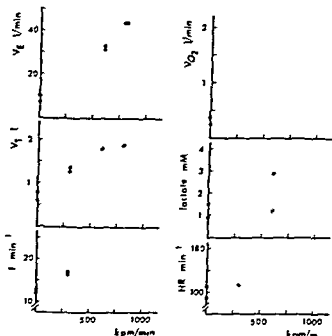


Fig 1 Steady state values of minute ventilation (V_E BTPS) tidal volume (V_T BTPS) and respiratory frequency (f) for subject NHB plotted against work load (kpm/min Open circles normal body temperature Filled circles high body temperature

Fig 2 Steady state values of oxygen uptake (V_O STPD) blood lactate concentration, and heart rate (HR) for subject NHB plotted against work load Open circles normal body temperature. Filled circles high body temperature

Temperature was registered continuously using a copper-constantan thermocouple placed at about 12 cm depth in the rectum and a calibrated monitoring instrument.

In the sessions in the climatic chamber body weight was measured before and after the warming up in the rest periods between work and at the end of the session (Table I) The subjects were allowed to drink freely (salted fruit juice) Arterial blood pressure was measured by the auscultatory method at rest and in each steady state of work One experimenter stayed in the chamber throughout each experimental session

The subjects were given a temperature vote on a dial (Wyon Andersen and Lundqvist 1972) and indicated in that way changes in their thermo comfort This signal as well as the rectal temperature and the dry temperature and the relative humidity of the chamber was monitored continuously

Results

Steady state data from the four subjects entering the study are shown in Table II and the data concerning ventilation tidal volume rate of breathing oxygen uptake blood lactate concentrations and heart rate from one male subject (subj NHB) are graphically presented in Fig 1 and 2

Expired minute volume at rest and at equal work loads was not substantially different at normal body temperature in comfortable surroundings and at elevated body temperature in the hot and humid chamber (Table II and Fig 1) Oxygen uptake on the other hand was lower at high than at normal temperature (the mean

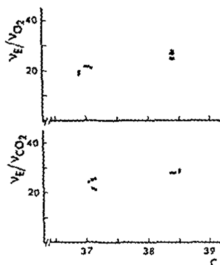


Fig 3 Results for all subjects of the ventilatory equivalent for O_2 (V_E/V_O) and for CO (V_E/V_{CO}) during exercise at normal and elevated rectal temperature

difference was 0 29 l/min) tested as paired observations at equal loads this difference was significant ($p < 0\,001$) Also at rest both O_2 uptake and CO elimination were usually lower at high than at normal temperature The ventilatory equivalent for O (V_E/V_{O_2}) was thus in all cases both at rest and during exercise significantly higher at high than at normal temperature ($p < 0\,001$) and similarly for the ventilatory equivalent for CO (*cf* Fig 3)

The mechanical efficiency of work conventionally calculated was consequently regularly higher at high than at normal temperature This however does not necessarily imply an increased mechanical efficiency on the part of the heated muscles In fact the total energy yield in hyperthermia may not have been substantially different from that at normal temperature as the increased blood lactate concentrations probably reflect an increased rate of lactic acid production The blood lactate concentration was higher at rest and started to rise further at a lower load than was the case at normal temperature (Table II Fig 2) Tested as paired observations the difference was statistically significant ($p < 0\,001$) Changes in the lactate/pyruvate ratio is regarded by some as a better index of the degree of anaerobic glycogen breakdown than changes in lactic acid concentrations alone (Keul Doll and Keppler 1968) The L/P ratio in hyperthermia exceeded that of normothermia significantly ($p < 0\,02$) at rest and during the two lowest work loads when the highest load too was included the differences between paired observations were not significant ($p > 0\,2$)

Inspection of Table II and Fig 1 shows that although expired minute volume was the same at the two levels of body temperature a definite difference in breathing pattern between the two conditions existed In hyperthermia ventilation was achieved at a higher respiratory rate and a comparably smaller tidal volume This pattern was a consistent finding in all subjects

TABLE II Combined results for each subject and each experimental condition (work load and rectal temperature). Ventilatory parameters, heart rate (HR), blood pressure (PP), blood concentrations of lactate (LA) and pyruvate (PA) and the lactate/pyruvate ratio (L/P). The conditions studied during the first experimental session at normal temperature have been marked * and those of the second †

Subject	Load kpm/min	Temp °C	V_E l/min (BTPS)	f min^{-1}	V_T ml (BTPS)	V_{O_2} l/min (STPD)
LJ	Rest	37.3	9.1	12.0	755	0.37
	Rest	37.3	5.0	11.5	450	0.21
	700	37.2	17.2	16.8	1025	0.90
	500*	37.2	22.3	15.5	1440	1.28
	700	37.4	31.9	17.0	1875	1.68
	900	37.5	43.2	21.4	2020	2.19
	Rest	38.4	4.5	10.2	440	0.18
	[illegible]00	38.6	21.1	26.5	795	0.76
	500	38.4	25.7	20.3	1265	1.05
	700	38.4	34.7	20.7	1675	1.42
HK	Rest	36.9	6.8	9.0	700	0.25
	Rest	37.1	7.0	7.4	950	0.28
	300	36.9	18.7	8.0	2340	1.01
	600	[illegible]7.1	26.2	12.0	2180	1.21
	800	37.4	42.1	17.0	2470	1.87
	1000	37.2	50.4	19.5	2580	2.28
	Rest	38.7	7.3	8.5	855	0.28
	300	38.6	15.0	13.0	1155	0.60
	600	38.6	32.0	15.5	2060	1.21
	800	38.6	34.5	20.5	1680	1.31
	Rest	37.1	10.9	16.6	655	0.32
	Rest	36.8	9.3	18.0	520	0.27
	300	36.8	23.6	24.0	980	0.95
	600	37.2	33.4	23.5	1420	1.39
	800	37.0	45.7	28.3	1610	1.95
	1000	36.5	53.6	29.4	1820	2.20
	Rest	38.5	9.6	20.3	470	0.27
	500	38.6	21.0	26.0	810	0.69
	600	38.5	30.1	26.0	1160	1.13
NHB	Rest	37.1	9.0	11.7	770	0.34
	Rest	37.1	11.0	13.7	800	0.39
	300	36.9	21.1	16.4	1285	1.07
	600	37.1	32.9	18.2	1810	1.56
	800†	37.0	43.7	23.3	1875	2.02
	1000	36.9	49.6	23.3	21[illegible]0	2.13
	Rest	38.8	7.3	12.0	600	0.24
	[illegible]00	[illegible]	23.4	17.0	1375	0.85
	600	38.4	31.5	20.2	1560	1.14
	800	38.5	43.5	24.8	1750	1.64

$\dot{V}_{CO_2}$ l/min (STPD)	$\dot{V}_E/\dot{V}_{O_2}$	$\dot{V}_E/\dot{V}_{CO_2}$	HR min⁻¹	BP mm Hg	LA mM	PA mM	L/P
0.27	24.6	33.7	96		1.1	0.10	11.1
0.15	23.8	33.3	94		1.9	0.23	8.3
0.77	19.1	22.3	—		1.0	0.12	8.1
1.07	17.4	21.9	148		1.6	0.23	6.9
1.52	19.0	21.0	164		3.2	0.18	17.6
1.98	19.7	21.8	—		6.4	0.24	26.7
0.14	25.2	32.0	147	115/[illegible]	2.3	0.30	7.6
0.61	27.8	34.6	142	100/50	2.6	0.29	9.1
0.86	24.5	29.9	180	140/60	3.2	0.38	8.6
1.34	24.4	25.9	186	140/70	4.7	0.26	17.7
0.22	27.2	30.9	80		0.7	0.11	6.9
0.24	25.0	29.2	76		1.4	0.09	15.4
0.86	18.5	21.7	100		0.7	0.10	7.0
1.09	21.7	24.0	120		1.4	0.09	15.0
1.68	22.5	25.1	136		2.6	0.13	20.0
2.25	22.1	22.4	148		3.7	0.15	24.0
0.23	26.0	31.6	96	120/70	1.6	0.16	9.8
0.52	25.2	28.9	120	120/60	2.0	0.14	14.0
1.13	26.4	28.3	138	140/50	3.2	0.21	14.8
1.21	26.3	28.5	150	150/50	5.2	0.39	13.3
0.27	34.1	40.4	106		1.0	0.17	5.8
0.23	34.4	40.4	84		1.3	0.10	13.1
0.89	24.8	26.5	108		0.9	0.17	5.5
1.31	24.0	25.5	102		2.1	0.11	19.2
1.84	23.4	24.8	124		2.9	0.13	22.1
2.13	24.4	25.2	144		3.1	0.21	15.0
0.22	35.6	43.6	124	100/60	1.8	0.14	12.6
0.59	30.4	35.6	148	110/60	2.7	0.19	13.8
1.04	26.6	28.9	165	120/60	4.4	0.27	16.1
0.28	26.5	32.1	86		0.9	0.21	4.3
0.33	28.2	33.3	92		0.9	0.11	8.2
0.81	19.7	26.0	104		0.8	0.23	3.5
1.31	21.1	25.1	120		1.2	0.11	11.3
1.63	21.6	26.8	136		2.4	0.17	13.7
2.06	23.3	24.1	156		3.7	0.29	12.8
0.18	30.2	40.3	110	120/70	1.5	0.16	9.3
0.75	27.2	31.2	112	120/70	1.5	0.15	10.1
1.11	27.6	28.4	140	140/60	2.9	0.19	15.1
1.55	26.5	28.1	170	150/60	3.3	0.22	14.8

a smaller range (Fig 3) The data at high temperature from our study are significantly higher than those in the normal temperature range both for O_2 and for CO_2 ($p < 0.001$) This implies the existence of some threshold in the temperature region of 38° C a conclusion substantiated by the absence of ventilatory changes in response to temperature rises of 0 6° C (Dejours *et al* 1958)

Direct measurements of alveolar or arterial pCO_2 and pH were not carried out Mean alveolar CO_2 concentrations could however be estimated from the rate of CO_2 elimination and the alveolar ventilations calculated using the physiological dead space/tidal volume data of Asmussen and Nielsen (1956) Thus estimated F_ACO_2 during exercise was 0 002—0 025 (average 0 01) lower in hyperthermia than in normothermia

The observed relative changes in blood lactate and in estimated alveolar CO_2 concentrations were subsequently used in a rough assessment of changes in arterial pH assuming an unchanged pH—log pCO_2 relationship at the two levels of temperature (*cf* Brewin *et al* 1955) It was also assumed that changes in base excess was caused by changes in lactate concentration only In no case were acidotic changes in pH observed at the high temperature

The increment in ventilatory equivalent found during work in hyperthermia is thus hardly caused by an increase in [H$^+$] stimulus It is consequently tempting to suggest that increased temperature constituted an additional ventilatory drive either *per se* or through interaction with other stimuli

Our study of ventilatory transients at the onset and end of work (Vejby Christensen and Strange Petersen 1973 a) gave little evidence in favour of any larger peripheral or central amplifying interaction of thermal and neural stimuli In a study of the ventilatory response to CO_2 and to hypoxia at normal and high body temperature (Vejby Christensen and Strange Petersen 1973 b) the results seem to indicate a possible temperature effect on the arterial chemoreceptors

Finally it might be argued that our experimental situation in the climatic chamber could involve activation of cutaneous receptors and/or other unknown factors related to these conditions of extreme thermal stress with possible effects on respiration

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References

ALEXANDER, S C R D WORKMAN and C J LAMBERTSEN Hyperthermia lactic acid infusion and the composition of arterial blood and cerebrospinal fluid *Amer J Physiol* 1962 202 1049—1054

ANDERSEN I and G R LUNDQVIST Design and performance of an environmental chamber at the University of Aarhus Denmark *Int J Biometeor* 1970 14 402—405

ASMUSSEN E and M NIELSEN Physiological dead space and alveolar gas pressures at rest and during muscular exercise *Acta physiol scand* 1956 38 1—21

BAZETT H C and J B S HALDANE Some effects of hot baths on man *J Physiol (Lond)* 1921 55 4—5 P

BERNTHAL T and W F WEEKS Respiratory and vasomotor effects of variations in carotid body temperature *Amer J Physiol* 1939 *127* 94—103

BREWIN E G R P GOULD F S NASHAT and E NEIL An investigation of problems of acid base equilibrium in hypothermia *Guy's Hosp Rep* 1955 *104* 177—214

CHAI C Y J Y MU and J R BROBECK Cardiovascular and respiratory responses from local heating of medulla oblongata *Amer J Physiol* 1965 *209* 301—306

COMROE J H The hypernea of muscular exercise *Physiol Rev* 1944 *24* 319—339

COTES J E The role of body temperature in controlling ventilation during exercise in one normal subject breathing oxygen *J Physiol* (Lond) 1955 *129* 554—563

CUNNINGHAM D J C and J L H O'RIORDAN The effect of a rise in the temperature of the body on the respiratory response to carbon dioxide at rest *Quart J exp Physiol* 1957 *42* 329—345

DEJOURS P A TEILLAC F GIRARD and A LACAISSE Étude du role de l'hyperthermie centrale moderee dans le regulation de la ventilation de l'exercice musculaire chez l'homme *Rev franç Étud clin biol* 1958 *3* 755—761

DILL D B H T EDWARDS P S BAUER and E J LEVENSON Physical performance in relation to external temperature *Arbeitsphysiolog* 1931 *4* 508—518

EULER C VON F HERRERO and I WEXLER Control mechanisms determining rate and depth of respiratory movements *Resp Physiol* 1970 *10* 93—108

GRIMBY G Exercise in man during pyrogen induced fever *Scand J clin Lab Invest* 1962 *14* (suppl 67) 1—112

GRODINS F S Analysis of factors concerned in regulation of breathing in exercise *Physiol Rev* 1950 *30* 220—239

HALDANE J S The influence of high air temperatures No I *J Hyg* (Lond) 1905 *5* 494—513

HEY E N B B LLOYD D J C CUNNINGHAM M G M JUKES and D P G BOLTON Effects of various respiratory stimuli on the depth and frequency of breathing in man *Resp Physiol* 1966 *1* 193—205

IAMPIETRO P F M MAGER and E B GREEN Some physiological changes accompanying tetany induced by exposure to hot, wet conditions *J appl Physiol* 1961 *16* 409—412

KEUL J E DOLL and D KEPPLER Zum Stoffwechsel de Skeletmuskels I Glucose Lactat Pyruvat und freie Fettsauren im arteriellen und venösen Blut der arbeitenden Muskulatur bei Hochleistungssportlern *Pflug rs Arch ges Physiol* 1968 *301* 198—213

LANDIS E M W L LONG J W DUNN C L JACKSON and U MEYER Studies on the effects of baths on man III Effects of hot baths on respiration blood and urine *Amer J Physiol* 1926 *76* 35—48

MORGAN D P F KAO T P K LIM and F S GRODINS Temperature and Respiratory Responses in Exercise *Amer J Physiol* 1955 *183* 454—458

NIELSEN M Untersuchungen uber die Atemregulation beim Menschen, besonders mit Hinblick auf die Art des chemischen Reizes *Skand Arch Physiol* 1936 *74* Suppl 10 83—208

OLSEN C An enzymatic fluorimetric micromethod for the determination of acetoacetate β hydroxybutyrate pyruvate and lactate *Clin chim Acta* 1971 *33* 293—300

SCHOLANDER P F Analyser for accurate estimation of respiratory gases in one half cubic centimeter samples *J biol Chem* 1947 *167* 235—250

TABATABAI M Respiratory and cardiovascular responses resulting from heating the medulla oblongata in cats *Amer J Physiol* 1972 *222* 1558—1564

VEJBY CHRISTENSEN H and E STRANGE PETERSEN Effect of body temperature on ventilatory transients at start and end of exercise in man *Resp Physiol* 1973a *17* 315—324

VEJBY CHRISTENSEN H and E STRANGE PETERSEN The effect of raised body temperature on the ventilatory CO_2 response in man *Bull Physio path* [illegible]

WHIPP B J and K WASSERMAN Effect of body temperature [illegible] exercise *Resp Physiol* 1970 *8* 354—360

WILLIAMS C G G A G BREDELL C H WYNDHAM N C STRYDOM J F MORRISON J PETER P W FLEMING and J S WARD Circulatory and [illegible] *J appl Physiol* 1962 *17* 625—638

WITZLEB E Über temperaturabhängige Aktionspotentiale [illegible] *Pflüg Arch ges Physiol* 1952 *255* 181—189

WYON D P I ANDERSEN and G R LUNDQVIST [illegible] discomfort during changes in the ambient temperature *J Hyg* [illegible]

Acta physiol. scand. 1973. 89. 352—358

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Effect of Albumin Concentration and Colloid Osmotic Pressure on Albumin Synthesis in the Perfused Rat Liver

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Abstract

DICH, J., S. E. HANSEN and H. I. D. THIEDEN. *Effect of albumin concentration and colloid osmotic pressure on albumin synthesis in the perfused rat liver.* Acta physiol. scand. 1973. 89. 352—358.

The effect of the albumin concentration and the colloid osmotic pressure on the rate of albumin synthesis was investigated in the perfused rat liver. The albumin concentration and the colloid osmotic pressure were changed independently. With the same concentration of albumin the rate of albumin synthesis decreased 20 to 30 per cent when the colloid osmotic pressure was increased by addition of gammaglobulin. It is concluded that the rate of albumin synthesis may be regulated by the colloid osmotic pressure. The albumin concentration seems to play a role in regulation only through the contribution to the colloid osmotic pressure.

The concentration of albumin in plasma is normally very constant, suggesting that the organism maintains an active regulation of albumin metabolism. Several factors, among these especially the albumin concentration and the colloid osmotic pressure have drawn attention as possible regulators of albumin synthesis (Rothschild, Oratz and Schreiber 1972). Plasmaphoresis giving rise to a reduced concentration of albumin results in an increased rate of synthesis, while infusion of albumin causes a decreased rate of synthesis (Andersen and Rossing 1967).

Liver perfusion experiments have shown that the rate of synthesis in some experiments is inversely proportional to the concentration of albumin or dextran in the perfusate (Rothschild *et al.* 1969, Tracht, Tallal and Tracht 1967). However it is not possible from these experiments to conclude whether it is the concentration of albumin or the colloid osmotic pressure in the plasma, which regulates the rate of synthesis as dextran is known to inhibit protein synthesis in the liver (Oratz, Rothschild and Schreiber 1970). The purpose of this investigation was to differentiate between the effect of albumin concentration and colloid osmotic pressure on the rate of albumin synthesis. We have therefore studied the effect of different protein fractions on the albumin synthesis in the perfused rat liver.

Materials and Methods

Animals Female Wistar rats weighing about 230 g were used The animals were fed *ad libitum* on a laboratory chow

Perfusion apparatus This was basically that described by Miller *et al* (1958) and Cohen and Gordon (1958) In order to minimize haemolysis during the perfusion oxygenation of the perfusate was accomplished in a 500 ml spherical flask rotating about 60 rev per min (Mortimore 1961) Gas exchange occurred between a gas phase of 95 % O and 5 % CO and a film of perfusate spread over the inner surface of the flask For the same reason as mentioned above a pulsatile pump made from transparent rubber tubing with two one way valves was used As pH decreased during the perfusion the perfusate was continuously titrated with 1 N NaHCO₃ from a pH stat (Radiometer Copenhagen) The liver was perfused under paraffin oil as described by Gordon (1968) In this way surface changes due to evaporation were avoided and the liver lobules were allowed to take up positions permitting maximum flow of the blood The perfusions were performed at a temperature of 37.5

Operative technique The surgical technique described by Miller *et al* (1958) was used In order to minimize anoxia the method was slightly modified After cannulation of the portal vein the inferior caval vein was immediately cut distally in the abdomen and infusion into the portal vein was started In this way the time during which the liver was without blood supply was reduced to about 1/2 min Hereafter the liver was isolated and transferred to paraffin oil The temperature and blood flow were allowed to stabilize for 30 min before start of the experiments

Perfusion medium As the rate of albumin synthesis was measured from the increment in rat albumin it was necessary to use heterologous blood Two different media were used The first consisted of bovine erythrocytes (washed 3 times with physiological saline) suspended in Krebs Henseleit buffer containing various amounts of bovine albumin (25 45 or 60 mg per ml perfusion plasma) amino acids as in the rat plasma (Scharff and Wool 1964) and glucose (15 mM) The hematocrit was 27—30 per cent

The second medium was composed of bovine erythrocytes as above and various amounts of bovine plasma and Krebs Henseleit buffer The ratio (vol/vol) between erythrocytes plasma and buffer were 1 3 2 2 and 2 4 0 respectively thus giving a final protein concentration of 18 36 and 72 mg per ml perfusion plasma

In order to change the protein concentration and thereby the colloid osmotic pressure without a simultaneous change in the albumin concentration bovine gammaglobulin 72 mg per ml was added to diluted plasma containing 18 mg and 36 mg protein per ml

Analytical methods The rate of synthesis was determined from the increment of the total amount of rat albumin in the perfusion volume The concentration of rat albumin was determined according to the method of Mancini Carbonara and Heremans (1965) using anti rat albumin Perfusion volume was measured by isotope dilution with [125]I albumin

Colloid osmotic pressure was measured according to Hansen (1961)* O₂ uptake was measured by estimation of the O₂ concentration in the medium entering and leaving the perfused liver (Laver *et al* 1965) From the difference in O₂ concentration and the actual flow rate the oxygen consumption was calculated

Dinitro synthesis In order to determine whether the increase in the concentration of rat albumin was due to synthesis and not only a washing out phenomenon cycloheximide (1 mM) was added to the perfusion medium in 2 expts The inhibitor was added 90 min after the start of the experiment

Results

Table I shows the results when a plasma free medium was used In the individual group the rate of albumin synthesis was constant during the first 2 h and declined significantly ($p < 0.05$) during the following hour Calculated from the first mentioned period a change in the albumin concentration from 60 mg per ml (group I) to 45 mg per ml (group II) which resulted in a concomitant decrease in the colloid osmotic pressure enhanced the rate of synthesis by 60 per cent $p < 0.001$ A

* The measurement of colloid osmotic pressure was kindly performed by Dr G Worring Institute of Medical Physiology A University of Copenhagen

TABLE I Rate of albumin synthesis in the perfused rat liver. The medium was composed of bovine erythrocytes, Krebs–Henseleit buffer, amino acids, glucose and varying amounts of bovine albumin. The results are given as the mean ± S.E. with the number of determinations in parentheses

Time	Rate of Albumin synthesis (mg/h per 100 g rat)		
	I Albumin 60 mg/ml *COP 30.[illegible] cm H_2O	II Albumin 45 mg/ml COP [illegible]0.8 cm H_2O	III Albumin 25 mg/ml COP 9.9 cm H_2O
30–90	1.6 ± 0.3 (5)	2.1 ± 0.1 (8)	1.8 ± 0.2 (6)
90–150	1.2 ± 0.3 (5)	2.4 ± 0.2 (8)	1.8 ± 0.3 (6)
150–210	1.0 ± 0.1 (5)	1.5 ± 0.2 (8)	1.1 ± 0.2 (6)
Mean 30–150	1.4 ± 0.2 (10)	2.2 ± 0.1 (16)	1.8 ± 0.2 (12)
t-test	I ~ II $p < 0.001$ II ~ III $p < 0.05$		

* COP Colloid osmotic pressure

further decrease in the albumin concentration to 25 mg per ml (group III) did not increase the rate of synthesis but resulted in a decrease of 18 per cent ($p < 0.05$) when compared to group II.

The results from the experiments with bovine plasma are given in Table II. In the individual group the rate of synthesis was constant in the period investigated. A decrease in the protein concentration from 72 mg per ml (group IV) to 36 mg per ml (group V) or 18 mg per ml (group VI) resulted in an increase in the rate of albumin synthesis by 38 per cent ($p < 0.01$) and [illegible] per cent ($p < 0.001$), respectively.

TABLE II Rate of albumin synthesis in the perfused rat liver. The perfusion medium was composed of bovine erythrocytes and varying amounts of Krebs–Henseleit buffer and bovine plasma

Time	IV Plasma Protein 72 mg/ml Albumin 32 mg/ml *COP [illegible]9 cm H_2O	V Plasma protein 36 mg/ml Albumin 16 mg/ml COP 12.0 cm H_2O
30–90	1.4 ± 0.2 (7)	1.9 ± 0.2 (6)
90–150	1.6 ± 0.1 (7)	2.1 ± 0.2 (6)
150–210	1.3 ± 0.2 (7)	1.9 ± 0.4 (6)
Mean	1.4 ± 0.1 (21)	2.0 ± 0.1 (18)
t-test	IV ~ V $p < 0.01$ IV ~ VI $p < 0.001$	V ~ VI $p < 0.01$

* COP Colloid osmotic pressure

Simultaneously the colloid osmotic pressure decreased from 30.9 to 5.1 cm H_2O. Thus the highest rate of synthesis (2.6 mg/h per 100 g rat) was obtained when strongly diluted plasma was used as perfusion medium.

Addition of bovine gammaglobulin to diluted plasma resulted in an increase in the protein concentration from 36 mg per ml to 108 mg per ml (group VII) and from 18 mg per ml to 90 mg per ml (group VIII), respectively. The albumin concentration was unaltered. The colloid osmotic pressure increased simultaneously to 29.0 and 16.1 cm H_2O, respectively. This resulted in a decrease in the rate of albumin synthesis by 30 per cent ($p < 0.01$) and 19 per cent ($p < 0.01$), respectively.

Oxygen uptake varied only slightly in the different experiments starting at about 8.0 μmol/min per 100 g rat which is equivalent with 2.1 μmol/min per g liver wet wt. During the perfusion the oxygen uptake decreased to 1.8 μmol/min per g liver wet wt at the end of the experiment. When an artificial medium containing 25 mg albumin per ml (group III) was used, the oxygen uptake was lower. In the first two hours the uptake was 1.7 μmol/min per g liver wet wt and decreased in the last hour to 1.2 μmol/min per g liver wet wt.

De novo synthesis. In two experiments with a medium as in group II cycloheximide (1 mM) was added after 90 min of perfusion. The rate of synthesis in the period from 150 to 210 min was only 5 and 9 per cent of the initial synthesis rate. This and the fact that the synthesis rate was nearly constant with time speaks in favour of a *de novo* synthesis.

Effect of modifications of the perfusion medium. As use of heterologous blood was necessary, erythrocytes and plasma from other species than cattle were tried as media. Washed erythrocytes from rat and sheep gave identical results and plasma from sheep did not change the rate of synthesis or the oxygen uptake.

In group VII and VIII gammaglobulin (72 mg/ml) was added to the medium as in group V and VI. Other experimental details as in Table I.

VI Plasma protein 18 mg/ml Albumin 8 mg/ml COP 5.1 cm H_2O	VII Plasma protein 108 mg/ml Albumin 16 mg/ml COP 29.0 cm H_2O	VIII Plasma protein 90 mg/ml Albumin 8 mg/ml COP 16.1 cm H_2O
2.5 ± 0.2 (6)	1.4 ± 0.2 (5)	2.1 ± 0.2 (7)
2.9 ± 0.3 (6)	1.1 ± 0.2 (5)	2.0 ± 0.2 (7)
2.4 ± 0.2 (5)	1.6 ± 0. (5)	2.2 ± 0.2 (7)
2.6 ± 0.1 (17)	1.4 ± 0.1 (15)	2.1 ± 0.1 (21)
V ~ VII $p < 0.01$ VI ~ VIII $p < 0.01$	IV ~ VII NS V ~ VIII NS	

Discussion

The present investigation shows that a change in the concentration of a colloid induces changes in the rate of albumin synthesis in the perfused rat liver

The experiments with plasma free medium (Table I) gave satisfactory results only when an albumin concentration of more than 25 mg per ml was used With the last mentioned medium it was not possible to continue the perfusion for more than 2 1/2—3 h as oxygen uptake and flow rate decreased considerably The reason for this is uncertain as diluted plasma with only 18 mg protein per ml (Table II) gave a stable perfusion with satisfactory flow rate and oxygen uptake With a synthetic medium containing more than 25 mg albumin per ml and with diluted or undiluted bovine plasma it was possible to continue the perfusion up to 5 h with only a slight decrease in albumin synthesis and oxygen uptake

The results with a synthetic medium containing 45 and 60 mg albumin per ml (Table I) and the results obtained with bovine plasma (Table II) showed that albumin synthesis could be regulated either by the albumin concentration or by the colloid osmotic pressure With a constant albumin concentration (8 or 16 mg per ml (Table II) it was possible to change the rate of synthesis by an increase of the colloid osmotic pressure (addition of gammaglobulin (Table II)) On the other hand a colloid osmotic pressure of about 30 cm H_2O resulted in the same rate of synthesis independent of the albumin concentration (16 and 35 mg per ml) Also the experiments with a colloid osmotic pressure of 12 0 and 16 1 cm H_2O (Table II) gave the same rate of synthesis irrespective of the fact that the albumin concentration varied by a factor 2 (16 and 8 mg per ml respectively) Fig 1 shows this significant correlation between the colloid osmotic pressure and the rate of synthesis From this we would conclude that the rate of synthesis in these experiments is regulated through the colloid osmotic pressure The albumin concentration seems to play a role in the regulation only through the contribution to the colloid osmotic pressure

In contrast to our results Katz Bonorris Okuyama and Sellers (1967) found that the rate of albumin synthesis in the perfused rat liver was independent of the pressure of a colloid (protein) However these authors found very high rates of synthesis (8—10 mg/h per 100 g rat) which is in contrast to the results obtained by others (Hoffenberg Gordon and Black 1971 John and Miller 1966) using the same methods Tracht Tallal and Tracht (1967) found that incorporation of [^{14}C] lysine into the perfused liver was nearly doubled when the albumin concentration in the perfusate was changed from 93 mg per ml to 2 mg per ml Similar results were obtained by Rothschild *et al* (1969) in the perfused rabbit liver Both of the last mentioned investigators added dextran to the perfusate and thus enhanced the colloid osmotic pressure without raising the albumin concentration The result was a decrease in the rate of synthesis Dextran is however known to inhibit protein synthesis to a considerable extent (Oratz Rothschild and Schreiber 1970) Moreover dextran gives rise to a leakage of enzymes out of the liver cells (Schimassek 1968) Such leakage is not seen if a plasma protein is used to increase the colloid osmotic pressure without raising the albumin concentration (Schimassek 1968)

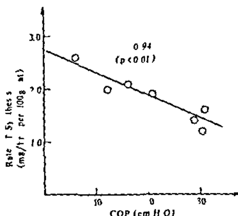


Fig 1 Correlation between colloid osmotic pressure (COP) and the rate of albumin synthesis in the perfused rat liver (The results from Table I and II were used in the calculation)

Data concerning the conditions in vivo are conflicting Already in 1943 Bjorneboe demonstrated in rabbits that hypergammaglobulinemia following hyperimmunization resulted in lowered concentration of albumin and he postulated an osmotic regulatory system Measurements of [^{131}] albumin metabolism in hyperimmunized rabbits showed however that the cause of the hypoalbuminemia was an increase in the plasma volume and not a decrease in the rate eof synthesis (Bjorneboe and Schwartz 1959) Rothschild *et al* (1962) carried out similar experiments and found a decrease in the rate of albumin synthesis The reason for this discrepancy could be the more pronounced hypoalbuminemia in the latter series of experiments Possibly the organism primarily regulates the colloid osmotic pressure by changing the plasma volume If this regulation is not sufficient resulting in a manifest increased colloid osmotic pressure the organism answers with a depressed rate of albumin synthesis

The lack of correlation between a hypoosmotic state and the rate of albumin synthesis as seen in several diseases *e g* nephrosis protein losing gastroenteropathies and cirrhosis of the liver (Rossing and Andersen 1966) could possibly be due to a limited capacity for increasing the synthesis

Although some of the results are controversial it seems fair to suggest both from the perfusion experiments and also from the hyperimmunization experiments that the colloid osmotic pressure plays a part in the regulation of albumin synthesis Other factors as hormonal balance and food and nitrogen intake have also been shown to be of importance (Dich Andersen and Rossing 1970 Rothschild Oratz and Schreiber 1972)

The skilled technical assi tance of Mrs Ing Damgaard i gratefull a knowledged

References

Andersen S P a d N Rossing Metabolism of album n and γ globul n during alb min infusion and duri g plasmapho esis S and *J clin Lab I est* 1967 0 183—184

Bjorneboe M Serum proteins during immunizat on *A ta path mi ob ol scand* 1943 20 221—239

BJORNEBOE M and M SCHWARTZ Investigations concerning the changes in serum protein during hyperimmunization. *J exp Med* 1959 *110* 259—2 0

COHEN S and N H GORDON Catabolism of plasma albumin by the perfused rat liver *Biochem J* 1958 *70* 544—551

DICH J S B ANDERSEN and N ROSSING Albumin metabolism in female rabbits treated with oral oestrogen-progestogen contraceptive Scand *J clin Lab Invest* 1970 *26* 31—34

GORDON A H Perfusion of the rat liver under paraffin oil. In *Stoffwechsel der isolierten perfundierten Leber* Editors W Staib & R Scholz. Springer Verlag Berlin 1968 pp 97—9

HANSEN A T., A self recording electronic osmometer for quick, direct measurements of colloid osmotic pressure in small samples. *Acta physiol scand* 1961 *53* 197—213

HOFFENBERG R. A. H GORDON and E G BLACK Albumin synthesis by the perfused rat liver *Biochem J* 1971 *122* 129—134

JOHN D W and L. L. MILLER, Influence of actinomycin D and puromycin on net synthesis of plasma albumin and fibrinogen by the isolated perfused rat liver *J biol Chem* 1966 *241* 4817—4824

KATZ J G BONORRIS S OKUYAMA and A. L. SELLERS Albumin synthesis in perfused liver of normal and nephrotic rats *Amer J Physiol* 1967 *212* 1255—1260

LAVER, M B A. I MURPHY A SEIFEN and E P RADFORD JR. Blood O_2 content measurements using the oxygen electrode. *J appl Physiol* 1965 *20* 1063—1069

MANCINI, G A O CARBONARA and J F HEREMANS Immunochemical quantation of antigens by single immunodiffusion *Immunochemistry* 1965 *2* 235—238

MILLER, L L. C G BLY M L WATSON and W F BALE The dominant role of the liver in plasma protein synthesis. A direct study of the isolated perfused rat liver with aid of lysine-C^{14} *J exp Med* 1958 *94* 431—452

MORTIMORE, G E. Effect of insulin on potassium transfer in isolated rat liver *Amer J Physiol* 1961 *200* 1315—1319

ORATZ M M A. ROTHSCHILD and S S SCHREIBER, Effect of dextran infusions on protein synthesis by hepatic microsomes. *Amer J Physiol* 1970 *218* 1108—1112

ROSSING N and S B ANDERSEN Colloid osmotic pressure and albumin metabolism. In *Protides of the Biological Fluids* Vol. 14 Editor H Peeters Elsevier Amsterdam, 1966 pp 319—322

ROTHSCHILD M A. M ORATZ and S S SCHREIBER, Albumin synthesis *New Engl. J Med.* 1972 *286* 748—757

ROTHSCHILD M A M ORATZ E. C FRANKLIN and S S SCHREIBER The effect of hypergammaglobulinemia on albumin metabolism in hyperimmunized rabbits studied with albumin-131I *J clin Invest* 1962. *41* 1564—1571

ROTHSCHILD M A. M ORATZ J MONGELLI and S S SCHREIBER, Effect of albumin concentration on albumin synthesis in the perfused liver *Amer J Physiol* 1969 *216* 1127—1130

SCHARFF R. and I G WOOL, Concentration of amino-acids in rat muscle and plasma. Nature (Lond) 1964 *202* 603—604

SCHIMASSEK, H Moglichkeiten und Grenzen der Methodik. In *Stoffwechsel der isolierten perfundierten Leber* Editors W Staib & R Scholz. Springer Verlag Berlin, 1968 pp 1—10

TRACHT M E. L TALLAL and D G TRACHT Intrinsic hepatic control of plasma albumin concentration. *Life Sci* 1967 *6* 2621—2628

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Thyroid Function Studies in Normal and Cold Exposed Rabbits Using ^{125}I

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Abstract

CARLSON L D H V ROOHK and O WILSON *Thyroid function studies in normal and cold exposed rabbits using ^{125}I* Acta physiol scand 1973 *89* 359—373

Normal uptake and retention of ^{125}I in thyroid was measured for 2—17 weeks in rabbits and the effect of TSH T_4 acute and extended cold exposure was studied There were marked interindividual variations in maximum uptake (mean 7 % range 2 5—18 %) in time to peak (12—72 h) in the slope of the retention curve and in changes in release rate Repeated doses gave different maximum uptakes but time to peak and form of retention curve remained essentially the same for each animal The mean biological half life for a 35-day period was 12 days for 8 rabbits the initial half life of around 9 days for the 1st week gradually increased to around 16 days during the last 3 weeks This change suggests a shift of the labelled iodine into thyroidal compartments with slower turnover rates Analysis of the multiexponential slope showed that the release phases for all rabbit were satisfactorily represented operationally by two exponentials The mean values for these corresponded to biological half lives of 6 4 and 21 1 days Neither acute and more severe (—3 and 6 C for 50 h) nor moderate extended cold (9—10 C for 7—16 days) had the slightest influence on the loss of radioiodine from the thyroid which nevertheless responded to small doses of TSH

Radioiodine has been used extensively for assessing thyroid function Usually it has been used to measure the thyroid uptake of the isotope only during the first few days after administration But many investigators have followed the thyroidal release rate of radioiodine over longer periods usually 2 to 3 weeks The release rate has been used as an index for assessing changes in thyroid activity imposed by various stresses such as cold This has been done in a variety of mammals including the guinea pig (Brown Grant and Pethes 1960 D Angelo 1960) hamster (Knigge 1960 Knigge 1963) rat (Brown Grant 1956 Cottle and Carlson 1956) and rabbit (Brown Grant *et al* 1954 a Brown Grant *et al* 1954 b Measurement of the release rate offers several advantages over measurement of simple uptake because it allows the determination of the biological half life of the radioiodine i e the length of time

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required for half of the isotope (corrected for decay) to disappear from the gland The rate of elimination of radioiodine after maximum uptake has generally been assumed to follow a single exponential curve with a linear trend in a semilogarithmic plot (Brown Grant 1956 Brown Grant *et al* 1954 a Brown Grant and Gibson 1955, French 1959 French 1966 Knigge 1960 Stevens *et al* 1955) Deviations from linearity have usually been considered as evidence for alterations in thyroid function (Brown Grant 1956 Brown Grant *et al* 1954 a, Knigge 1963 Knigge *et al* 1957)

In previous studies the isotope of choice has been ^{131}I with a physical half life of 8 05 days This has limited the length of time during which the release rate can be followed By using ^{125}I with an approximate 60 day half life (Myers and Vanderleeden 1960) the retention curve of radioiodine can be measured over a considerably longer period allowing for a more thorough analysis of the biological half life of iodine in the thyroid ^{125}I has the added advantage of a low energy gamma emission and the absence of beta particles Radiation exposure is therefore appreciably less than with ^{131}I in spite of its longer half life (Daniel *et al* 1962 Harper *et al* 1963 Myers and Vanderleeden 1960 Porath *et al* 1966 a) The disadvantage is the greater extent of tissue absorption This may to a large extent be counterbalanced by backscattering from tissues surrounding the thyroid There seems to be a fairly close agreement between uptake values obtained with ^{125}I and ^{131}I as shown by double isotope experiments (Porath *et al* 1966 b Wood *et al* 1963 Wilson *et al* unpublished data)

This study was designed to investigate the nature of the thyroid retention curve in rabbits by measuring the thyroidal ^{125}I content for an extended period after a single injection under normal conditions and how the slope of the curve is influenced by experimental alterations in thyroid secretion It will allow the assessment of some of the physiological variables that may affect thyroid activity in rabbits However it should be emphasized that this is an indirect method of assessing thyroid function awaiting more direct methods to measure the thyroid hormone secretory rate It is greatly hampered by the slow release of injected radioiodine requiring several weeks More rapid and transient changes of the secretion of thyroid hormone can however be read off as disturbances of the regular disappearance curve

Procedure

Ten male and 1 female (R5) pedigree Dutch rabbits age about 1 year mean body weight 2 ? kg range 1 8—2 8 kg) housed in separate cages were used Three pairs (no R2 R3 R6 R7 and R8 R3) were litter mates The animals were fed *ad lib* on a Purina diet (No 5515C) and tap water throughout the experiments They normally consumed 90—105 g food per day Analysis showed that the total stable iodine content of the food was 65—70 μg/100 g dry pellets of this amount about 50 μg/100 g consisted of protein bound iodine All except one (R1) had been kept on this diet for well over 4 months before the start of the experiment since changes in the slope of the thyroid release curve have been shown to occur during the 1st 2—3 months after arrival of the animals to the laboratory attributed to changes in the dietary iodine intake (Brown Grant *et al* 1954 a The rabbits were kept in a temperature controlled room at 2? C (±1 C) except when cold exposed The temperature during the moderate cold exposures varied between 8 and 11 C This difference (approx 12 C) between the control

and cold environments was chosen to allow comparison with a similar earlier study by Brown Grant *et al* (1954 a) Except for temperature the cold room facilities were the same as those of room temperature The rabbits were moved with their cages into and out of the cold room. Air movement in the cold was minimal A 12 hour light dark regimen was instituted at 0700

The normal uptake and retention curve of radioiodine was studied for periods ranging from 12 to 190 days (usually 35 to 50 days) in 18 experiments on 11 normal rabbits Six of these animals were used more than once In six experiments the measurements were made at shorter time intervals during the first few days to follow the early uptake change In certain cases urine was collected and measured to estimate the amount of radioiodine excreted

In one anesthetized rabbit the retention of radioiodine in the gland was first measured external to the skin then with the thyroids surgically exposed *in situ* and thereafter with the covering tissues replaced so as to approach the presurgical state Finally the thyroids were excised and measured *in vitro* in a polyethylene tube of the same quality as that of the standard and placed in the same polyethylene holder The count of the excised thyroids (weight 0 35 g) in the plastic holder was practically identical to the presurgical count *in vivo* showing that the standard tube and holder closely approximated the net result of attenuation and backscatter by the tissues surrounding the thyroids The count of the surgically exposed thyroids was significantly higher (+18 %) demonstrating a high amount of backscatter which to a large extent counterbalances the loss by attenuation in the covering tissues

In 3 expts 3 to 4 intraperitoneal injections of thyroxine (50 to 100 μg L-thyroxine sodium salt B grade Calbiochem Los Angeles Calif) were given each animal at 2 to 3 day intervals In 9 expts. TSH (0 8 to 3 7 IU lyophilized bovine TSH B grade Calbiochem Los Angeles Calif) was injected s.c. on 1 or 2 consecutive days

Seven rabbits were exposed to moderate cold [illegible] at 9±1 C for 7 days and three at 10±1 C for 16 days Two other rabbits were submitted to a short cold stress of 50 h at 6 and −3 C respectively

Methods

For each experiment the rabbit was given a single injection of [illegible] I which has a physical half life of 57 4 to 60 2 days (ICRP 1964 Matthews 1960 Myers and Vanderleeden 1960) The amount administered usually 4—9 μCi, was given in a volume of 0 5—1 ml sterile saline The dose was counted in the syringe prior to its injection in the marginal vein of the ear and the activity remaining in the empty syringe was subtracted from the original count. Residual activity from a previous experiment was present in only one case (R3B) and this was corrected for in the maximum uptake measurement

The counting was carried out with a collimated solid scintillation detector having a 2 inch wide by 2 mm thick NaI (Tl) crystal with a 0 025 mm aluminum radiation entrance window and a current base with a low impedance output possessing a resolution time of 1 [illegible]s With the counting rates used coincidence losses were so small as to be negligible The collimation consisted of heavy copper tubing with an 8-mm thick wall The recommendations given by IAEA (1967) regarding collimation and field of vision (modified for rabbit size were closely followed The analyzer/scaler used was a Baird Atomic model 530A spectrometer with a digital-to-analog output which allowed daily calibration of the unit by printing out the isotope spectrum on a Varian strip chart recorder The scale was calibrated fo[illegible] 250 kev full scale base The threshold was set at 7[illegible] with an [illegible] window which straddled the [illegible] 35 4 kev peak, excluding the K escape peak and the coincidence [illegible] Background counts for this unit consistently remained around 16 [illegible] per min and in no instance exceeded [illegible]5 counts per min

A minimum of 100 000 total counts were alway[illegible] the standard [illegible] reducing the standard error of any given count to 3 SF < 1 [illegible] of each standard was plotted over th[illegible] experimental period [illegible] of the log values for the longest measured standard at [illegible] ±0 1 days for [illegible] I over a 115 day period The slope was [illegible] thereby assuring no drift in calibration nor change in [illegible] mental period A total of from 20 000 counts at the [illegible] for each uptake measurement on [illegible] rabbit [illegible] on each counting occasion As the number [illegible] and biological turnover the preset number [illegible] stepwise from 20 000 to 10 000 to 5000 to 2500 to 1[illegible]0 and finally [illegible] adhered to throughout all experiments and allowed [illegible] changes in the standard error of the measurement [illegible] as long as 10 min or more for a given measurement Usually the animal was [illegible] extensive [illegible]

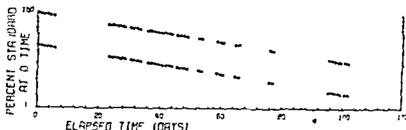


Fig 1 Semilogarithmic plot of longest measured standard of ^{125}I at 20 cm (top) and 30 cm (bottom) distance showing a linear decay rate with a physical half life of 59 9±0 1 days

zation since by this time it was very familiar with the procedure Extended periods of restraint are said to affect thyroid function in unaccustomed rabbits (Brown Grant *et al* 1954 a)

During the thyroid uptake measurements the rabbit was placed in a modified commercial rabbit restrainer (Model Ra-4 Aerospace Industries Inc.) and its head and neck were immobilized over a 40-mm diameter opening in a 3 mm thick lead plate (Fig 2) Another lead plate shielded the bladder of the animal A numbering index for the position of the nose in the restrainer aided in reproducing the correct position for each rabbit The opening in the lead plate was adjusted to leave only the thyroid region exposed for counting which was done with the collimated probe precisely positioned at 20 and 30 cm distance from crystal to the closely clipped skin surface Counting was performed twice at each distance on every occasion In a few cases the distances were reduced to 10 and 20 cm thereby increasing the number of counts obtained to enable the retention curve to be followed over an extended period This was usually done when the time to reach 1250 or 625 counts exceeded 5 min at the 30 cm distance

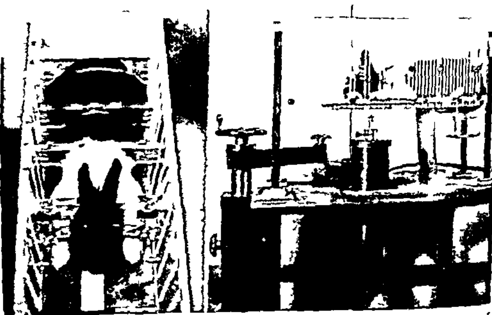


Fig 2 Unit for restraining rabbit during thyroid uptake measurements Left Top view of restrainer showing rabbit in counting position with nose at numbering index Right Side view showing collimated probe with positioner rod in place ready to be raised through hole in sliding lead plate that covers area surrounding thyroid Vertical lead plate at right shields hind part of rabbit, including urine collection bottle (not shown) and feces collector

Measurements carried out at all four distances on the same occasion disclosed no differences in the results obtained with either pair of distances Distances were closely checked before and after each measurement by utilizing positioner rods (Fig 2) The standards were counted at the same distances in a polyethylene holder which closely simulated attenuation and geometry in the rabbit The dosing syringes also fitted into this holder

A ratio between the counting activity at the 20 and 30 cm distances (as well as at 10 and 20 cm) for both standard and rabbit was established Counts causing an appreciable deviation in the ratio were checked for the purpose of discovering positioning inconsistencies if necessary a recount was made A series of 10 consecutive uptake measurements were made on one of the rabbits at each distance Between each measurement the animal was completely removed from the restrainer and then replaced and repositioned The differences between these measurements were no greater than the normal random error of counting Computer error analysis of the duplicate measurements at both 20 and 30 cm distances (as well as at 10 and 20 cm) demonstrated that the standard error between two consecutive measurements at any distance or at any level of counts was always very close to the theoretical random counting error at each particular level of counts Indeed it usually tended to be less which is consistent with the fact that the theoretical error was calculated for the minimum number of counts at that particular level while the actual number of counts obtained was normally somewhat greater than the preset count In view of this the possible additional error introduced by slight variations in positioning of the animal can be disregarded for all practical purposes The standard error of the total number of counts from all four measurements is therefore representative for each point of the retention curve With declining radioactivity in the thyroid the standard error increased from 0 35 % (at 4 × 20 000 counts) at the start to 2 0 % (at 4 × 625 counts) at the end of a retention curve. The counting error for all parts of each experiment was thus consistently less than half of the usually accepted 5 % For any measurement on the retention curve ±3 S E is so small that it does not exceed the size of the symbol for each measuring point in the diagrams

Analysis of variance also demonstrated that there was a slight but statistically evident difference between measurements made at the two distances used each time This difference may be due to the distance from the skin to the thyroid not being exactly the same in the individual rabbits and consequently also slightly different from the predetermined distances to the standard The shorter the measuring distance to the skin the greater the effect of this variation in thyroid distance on the uptake measurements which becomes apparent as a statistical difference between the measurements at the two distances This variation also showed up in the individual ratio between the counting activities of the two distances each rabbit having a somewhat different mean ratio suggesting variations in the depth of localization of the thyroids in different rabbits

In 3 expts the rabbit was catheterized with a sterile Bard Foley catheter size 8 Fr with a 3 ml balloon) for the purpose of urine collection During the entire 48 hour collection period the rabbit was kept in the restrainer which allowed the urine to flow freely into a polyethylene bottle Only the hind portion of the animal was restrained while allowing free access to food and water during this period Feces were collected separately in a container underneath the restrainer but were not analyzed

Four ml aliquots of urine samples in thin walled polyethylene tubes were counted in a heavily shielded well scintillation detector (Baird Atomic model 810 LX with a 3 by 3 in h w ll crystal with 0 001 inch aluminum radiation entrance window) Background was consistently 7—9 counts per min

Results

Maximum thyroidal uptake of ^{131}I in percent of administered dose approximate time to reach the maximum and biological half lives (T_B) are presented in Table I T_B have been calculated for the initial part and for the tail end of the downward slope of the retention curve (column E and F Table I) using a linear regression analysis of the log values for those parts of the curve that could be fitted to a straight line Mean T_B (column D Table I) for 1st 3 weeks of the downward slope of the curve has been estimated from two points only (start and end of interval) The retention of radioiodine in the thyroid with respect to time for each animal is shown in Fig 3 to 6 The rabbits are numbered R1 to R11 the letters A B C and

TABLE I Administered dose of ^{131}I, maximum uptake, time to peak, and biological half-lives (T_B) of radioiodine in the thyroid of Dutch rabbits

Rabbit No	A µCi	B	C hrs	D days	E days ± S.E. (day)	F days ± S.E. (day)
R 1	5.9	18.3	72	11.8	10.6 ± 0.8 (3 to 15)	14.6 ± 1.7 (24 to 35)
R 2A	7.0	17.8	48	11.2	10.5 ± 0.5 (2 to 11)	15.1 ± 2.2 (25 to 41)
R 2B	8.0	5.0	48	11.6	8.8 ± 1.1 (2 to 10)	15.5 ± 1.8 (24 to 37)
R 3A	4.4	3.7	< 12	—	8.6 ± 1.1 (2 to 7)	— —
R 3B	12.3	4.5[2]	< 12	11.3	7.6 ± 0.9 (2 to 6)	13.1 ± 0.5 (13 to 37)
R 3C	4.0	2.5[2]	—	—	10.4 ± 1.0 (2 to 10)	17.8 ± 2.9 (18 to 34)
R 4A	9.0	6.1	48	21.2	27.6 ± 5.6 (2 to 10)	31.9 ± 3.2 (81 to 120)
R 4B	4.0	4.1	48	—	21.0 ± 2.1 (? to 10)	— —
R 5[1]	10.0	5.8	24	17.0	14.4 ± 2.3 (1 to 7)	24.0 ± 1.4 (34 to 63)
R 6A	10.0	5.3	12	10.5	7.6 ± 2.1 (? to 10)	17.9 ± 2.7 (23 to 38)
R 6B	8.0	3.4[2]	—	11.3	7.9 ± 0.5 (2 to 10)	16.3 ± 1.7 (21 to 37)
R 7	10.0	6.6	24	9.1	5.4 ± 0.5 (1 to 8)	14.6 ± [illegible]3 (21 to 44)
R 8A	9.0	3.7[2]	—	—	4.9 ± 0.2 (2 to 11)	— —
R 8B	3.9	2.5	24	—	4.8 ± 1.9 (1 to [illegible])	—
R 9A	9.0	5.5[4]	48[4]	8.9	7.1 ± 0.3 (2 to 11)	13.8 ± 1.0 (28 to 48)
R 9B	4.0	3.1[4]	48[4]		8.3 ± 1.7 (2 to 10)	—
R 10	8.4	7.4	48[4]	10.3	8.3 ± 0.7 (2 to 11)	17.9 ± 1.3 (30 to 49)
R 11	11.3	[51.6]	[138]	[8.8]	[5.3 ± 0.3 (10 to 21)]	[13.2 ± 1.1 (31 to 44)]
Mean[5]		7.1	36	12.1	9.[illegible] (2 to 10)	16.3
Median[5]		5.8	36	11	8.6 (2 to 10)	15.5

A = Administered dose of ^{131}I (µCi)
B = Max uptake (% of administered dose)
C = Approx. time to max uptake (hrs)
D = Mean T_B (days) for 1st 5 weeks of downward slope
E = T_B ± 3 S.E. (days) for the initial downward slope of the early period (day a to day b)
F = T_B ± 3 S.E. (days) for the tail end of the slope (day c to day d)
[1] R5 is the only female rabbit
[2] Corrected for remaining activity of previous dose
[3] Extrapolated value
[4] May have peaked earlier with a higher maximum
[5] The mean and median have been calculated from means of each rabbit, excluding extrapolated values. The extreme values of R 11 within brackets have not been included. N = 10 for columns B, C, F; N = 9 for columns D, E

different experiments with a repeated dosing of the same rabbit. From the data it is immediately evident that there are striking individual differences in the maximum uptake (range 2.5 to 52 %), in the time to reach maximum (< 12 to 138 h), and in the slope of the retention curve. Fig. 3 illustrates these large interindividual variations. *R11 is unique in* having by far the highest uptake (52 %) and the longest time to peak, with a dome shaped plateau for the 1st 10 days before the thyroid release rate markedly increased. R3, on the other hand, reached maximum within 12 h, while R8 had the lowest uptake, only 2.5 %, and the shortest initial T_B (5 days). R4 is exceptional in exhibiting an almost flat uptake plateau for the 1st 5 days and an unusually long overall T_B (Fig. 4), which made possible measurements up to 120 days.

As seen in the various figures, T_B varies greatly interindividually as well as intra

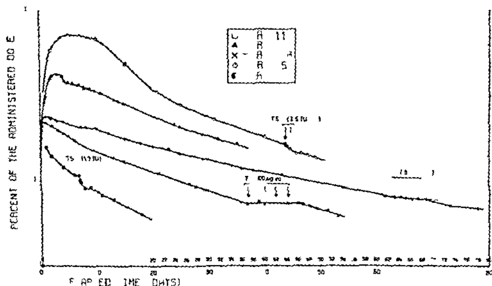


Fig 3 Individual thyroidal retention curves showing the great variability in maximum uptake time to peak and release slope The effect of T_4 and TSH injections on the slope of the retention curve is clearly evident ±3 S E for each observation is so small that in the diagram it does not exceed the size of the symbol for each measuring point of the curve

individually and also between litter mates The mean and median T_B for 8 rabbits (excluding the most extreme one) over a 35 day period was found to be 11 days when the individual changes in release rate of the retention curve are disregarded But the slope of the individual curve conforms to no consistent pattern although multiexponential in form The average initial T_B for the 1st week was around 9 days but then gradually increased to around 16 days during the last 3 weeks of the curve The range however was great 5 to 28 days for the initial slope after

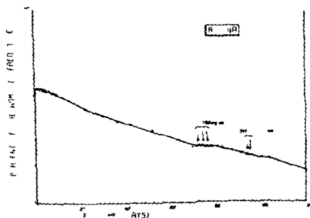


Fig 4 Thyroidal retention of I in the longest measured rabbit (120 days) showing the effect of injections of T TSH

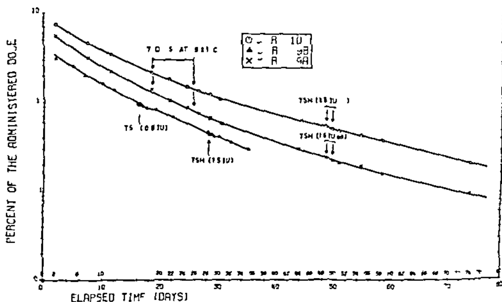


Fig 5 Effect of moderate cold exposure and TSH on thyroidal retention curve

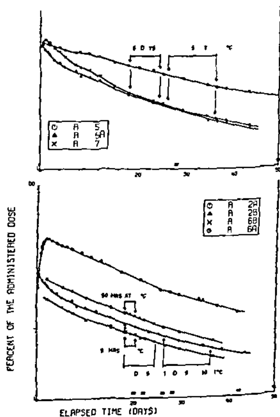


Fig 6 Effect of extended moderate cold exposure and acute more severe cold on thyroidal retention of ^{131}I

maximum uptake and 13 to 32 days for the tail end depending how far in time it had been measured It should be noted that reaccumulation of labelled radioiodide from degraded thyroid hormone has not been taken into account since the thyroid was not blocked after maximum uptake to avoid the possibility of artifactual results due to blocking It is estimated that only about 10 % of the labelled hormone eventually recirculates to the gland (Brown Grant *et al* 1954 a)

Definite irregularities in the early phase of the retention curve were noticeable during the first 8—10 days as shown by R1 and R5 (Fig 3) Similar tendencies were noticed in R5 R6 and R7 (Fig 6) If a rabbit was dosed a second time after previous radioactivity had been eliminated it showed a different degree of maximum uptake (*cf* Table I) but the time to peak and the form of the retention curve remained very much the same (*cf* Fig 5 6)

The 48 hour collections of urine showed that the sum of the amount of radioiodide excreted in the urine and the maximum amount retained in the thyroid exceeded 90 % (R4 90 6 % R2A 91 4 % R3B 92 4 %) indicating that relatively little of the dose was lost in the feces less than reported by Brown Grant and Gibson (1955)

Rabbit R3B (2 0 kg) was given four injections of T_4 (100 μg each) at 2 to 3 day intervals (Fig 3) R4A (2 8 kg) received 3 injections of T_4 (100 μg each) at 2-day intervals (Fig 4) and R5 (1 8 kg) had three injections of T_4 (50 μg each) with 2 day intervals (Fig 3) In each of these three cases the retention curve immediately plateaued and became parallel to the base line 2—4 days after the last injection the retention curve again resumed the same release slope as prior to the injections This was regarded as a normal feedback inhibition of thyroidal secretion Total inhibition for 3—4 days was achieved by 100 μg T_4 every 2nd day (*cf* R3B and R4A) while the effect of 50 μg T_4 appeared to be less complete not lasting more than about 2 days (*cf* R5) These results agree with the previous observations by Brown Grant *et al* (1957 1954 a)

Varying amounts of TSH were injected in 6 rabbits in 9 experiments (Table II)

TABLE II Injections of TSH and degree of response

	I					II			
Rabbit No	R 8B	R 3C	R 9B	R 4B	R 9B	R 11	R 9A	R 10	R 4A
Body weight (kg)	2 6	2 0	2 4	2 5	2 4	1 9	2 4	2 1	2 8
Day of injection after I dose	8	14	16	21	28	44—45	48—49	48—49	94—95
TSH (IU)	1 7	1 2	0 8	3 7	1 5	2 5+2 5	1 6+1 6	2 5+2 5	1 6+1 6
Clear TSH response	++	+—	—	—	(+	++	—	—	—
Secondary inhibition	++	++	—	—	+	++	—		+
See Figure No	3				5	3	5	5	4

Injections were either single (I) or given on 2 subsequent days (II) Standard activity of bovine TSH = 0 74 IU/mg

In only 3 of these (R8B R3C R11) were clear responses obtained with a temporary increase in the loss of radioiodine from the gland (*cf* Fig 3) while the response in 2 others (R4B R9B) was less clear (*cf* Fig 5) However even if a clear response was not seen a more or less pronounced inhibition in the release of radioiodine was usually observed several days after the TSH injection, increasing the T_B for a considerable number of days There was no correlation in degree of TSH response with amounts of TSH injected number of injections, or the number of days following the administration of radioiodine It seemed more to be a question of sensitivity of the individual rabbit to the bovine TSH than a matter of dose response or timing since the three rabbits that did not give a clear response did not do so even after repeated injections at different times

No effect of cold exposure on the retention of radioiodine in the thyroid was observed Neither moderate cold over a longer time (7 days at 9 ± 1° C and 16 days at 10 ± 1° C) nor acute severe cold (6 and −3° C for 50 h) had any noticeable influence on the slope of the retention curve (Fig 5 and 6) Uptake measurements made every 2 h for the 1st 8 h disclosed no change in release rate of radioiodine from the gland (Fig 6)

Discussion

With the exception of one rabbit with an unusually high uptake value of 52 % for which there is no explanation the uptake is quite low with a mean of 17 % This agrees with other investigations French (1959 1966) reported mean uptakes around 7—12 % for Dutch rabbits Taurog *et al* (1964) found uptakes in New Zealand rabbits to be 5 to 8 % and Brown Grant and Gibson (1955) reported 5 to 10 % in their rabbits The great interindividual variation (range 2 5 to 18 3 %) seen in the present investigation was likewise observed by French (range 2 5 to 11 3 % personal communication) as was the variability to reach maximum (Brown Grant *et al* 1954 a French 1959 Taurog *et al* 1964) The interindividual range in biological half life is also great especially during the initial period French found a T_B of 5 to 11 days for about 2 weeks (French 1959) and 5 to 24 days (mean 16 days) for 3—4 weeks (French 1966) His release curves however were plotted as single exponential slopes and the longer T_B found by French for the 4 week period is no doubt due to the multiexponential character of the slope demonstrated by the present investigation the T_B increasing with time after dosing The higher the uptake or the larger the dose the longer the release curve can be followed The longer it is followed using ^{131}I the longer the observed T_P will tend to be when assuming a single exponential function

The degree of intraindividual variation in maximum uptake upon repeated dosing observed in the present investigation in spite of the carefully controlled food and environmental conditions is more surprising Variations in water intake and kidney excretion may have affected the uptake There seemed to be a tendency for lower uptakes to occur during the second test which may possibly be attributed to a voluntary restriction in water intake during the first test because the rabbits at that time

were unused to or disturbed by the experimental procedure Changes in water intake has been shown to affect the radioiodine uptake (French 1966 Storaasli *et al* 1953)

In view of the large interindividual differences in uptake and general appearance of the retention curve of radioiodine one rabbit cannot be used as control for another in this type of measurements Each rabbit has to be his own control but maximum uptake in itself cannot be used because of its inconsistency The best reproducible parameter seems to be the T_P and the shape of the individual retention curve This slope is a sensitive indicator of changes in the release rate of radioiodine from the thyroid as demonstrated by the injections of small amounts of T_4 and TSH The maximum dose of TSH given in this investigation was 8 times smaller than the largest used by Brown Grant *et al* (1957) Not all rabbits responded to the TSH doses administered in the present investigation Since TSH is relatively species specific it is possible that the rabbits were not very sensitive to the bovine TSH used Bovine TSH preparations have been shown to produce antisera capable of neutralizing the TSH activity (Werner *et al* 1961) In a similar study of the Beagle dog (Wilson *et al unpublishesd data) marked responses were observed with as little as* 0 5 IU bovine TSH Further a low potency of the first batch of TSH used cannot be excluded since none of the rabbits (R4A R9A R10) injected with this batch showed any response in spite of 2 subsequent injections

The fact that some TSH injections did not evoke a clear response with an increase in the relea e rate but nevertheless exhibited signs of a secondary inhibition indicates that the amounts used were at or near physiological levels In the three rabbits demonstraing a clear TSH response the inhibition was as complete as that following the injection of T_4 in agreement with the results reported by Brown Grant *et al* (1957 1954 a 1955) This inhibition occurs very shortly after T_4 but it requires at least 24 h for inhibition after TSH administration as shown in humans by Greer and Shull (1957) This plateauing of the release curve after the cessation of the TSH response most likely represents transient inhibition of the endogenous output of thyrotropin

Although the individual relea e curves vary considerably two main types of curves may possibly be discerned In one typified by R2 and R4 (Fig 6 and 4) the slope remained approximately linear for perhaps 3 weeks and then rather suddenly changed to a new linear slope In the other typified by R9 and R10 Fig 5) the slope curved rather evenly the whole time approaching linearity at the end The increase in T_B typical for these multiexponential curves gives evidence of a gradual change in secretion rate which suggests a shift of the labelled iodine into compartments with slower turnover rates or a more rapid disappearance of labelled iodine from a pool with a faster turnover rate Thyroid uptake data obtained from all rabbits were analyzed by least squares fitting to sums of exponential (Berman *et al* 1962) using the Berman Weiss computer program SAAM on a UNIVAC 1108 computer (Berman *et al* 1968) In all cases the relea e phases were satisfactorily represented operationally by 2 exponentials which described spaces of distribution of

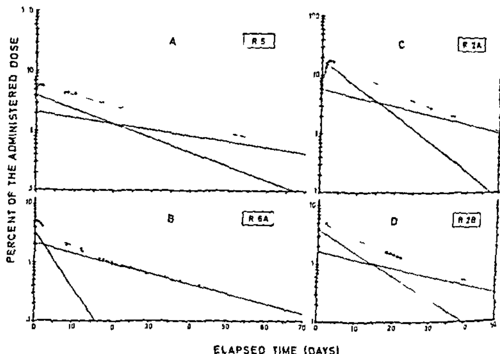


Fig 7 Thyroidal radioiodine retention data fitted to sums of exponentials by a least square procedure Solid lines = separately calculated exponentials Broken lines = calculated curve (sum of exponentials) Open circles = original data

150 and 230 ml and fractional rates of turnover of 0 130 and 0 0344 per day respectively (Barandes and Wilson unpublished data) It is interesting to note that all the individual release curves were sufficiently described by only two exponentials in spite of their great variation in appearance The slope of the final component appeared to show a relatively moderate variation among individual rabbits suggesting that the slow phase represents a fairly constant biological turnover of iodide in the thyroid

TABLE III Exponentials (cf Fig 7) representing 2 fractional release rates for each rabbit expressed in terms of biological half life (T_B) of thyroidal iodine (cf Table I)

Rabbit No	Fractional release rates (T_B days)	
R 1	5 1	17 0
R 2A	6 3	20 5
R 2B	7 5	21 0
R 4A	10 9	23 1
R 5	12 6	31 0
R 6A	3 2	17 5
R 6B	3 2	15 5
R 7	4 9	18 6
R 9A	5 9	18 3
R 10	6 6	25 4
R 11	4 1	18 5
Mean	6 4	21 1
S D	±3 0	±5 1

in the population The faster component is more variable (Fig 7, A—C) affecting the shape of the final slope inversely with the rapidity with which it is complete thereby characterizing the individual form of the release curve As seen in Fig 7 C—D and Table III repeated experiments on the same rabbit (*e g* R2 R6) will give reproducible slopes for both exponentials which thus seem to have a charac teristic combination for each rabbit Since the effect of recirculated iodine from de iodination of labelled thyroid hormone is not accounted for in these calculations slightly more rapid rate constants would be expected to reflect the true rate of thyroid iodine turnover than obtained by fitting to sums of exponentials Analyses of the entire iodine metabolic system would be necessary to determine the best estimates for these rates and have been carried out in man (Berman *et al* 1968) and in dogs (Belshaw Barandes Becker and Berman unpublished data) A discussion of the effect of recirculated iodine has been carried out by several authors (Rall *et al* 1964 Berman 1972) Where maximum thyroid uptake is 10—20 % as in these rabbits a change in the rate constants as a result of recirculation is not likely to be greater than 10 % In Table III are shown the two fractional rates of turnover for each rabbit expressed in terms of biological half life of thyroidal iodine in different spaces of distribution for comparison with the biological half lives shown in Table I and representing the sum release rate It may be noted that the female rabbit has the longest fractional release rates Previou studies have only covered the first few weeks and have thus not demonstrated the observed change in T_B The definite ir regularities in the early phase of the retention curve may also be related to such shifts into different intrathyroidal pools affecting the specific activity of the secreted thyroid hormone

In these experiments neither acute severe nor moderate cold over a longer time had any influence on the retention of radioiodine in the rabbit thyroid No sign of a change in T_P or an inhibition in release rate as with TSH was observed that might suggest a cold stimulated TSH discharge This is in contrast to several studies show ing in the rat (Itoh *et al* 1966 Konno and Koseki 1967) rabbit (Bottari 1957) and hamster (Knigge *et al* 1959) an increase of TSH secretion in response to acute cold exposure reaching a peak within 2 h and then rapidly declining It has also been repeatedly shown that exposure of rats to moderate cold stimulates the thyroidal uptake of radioiodine and increases the discharge from the gland and that short term exposure to cold is more effective in stimulating the thyroid than prolonged exposure (Pitt Rivers and Tata 1959)

In the rat (Cottle and Carlson 1956 Straw and Fregly 1967) hamster (Knigge 1963 Knigge *et al* 1957) and guinea pig (Stevens *et al* 1955) T_B of thyroidal radioiodine is markedly shortened by cold exposure However several investigations (Galton and Nisula 1969 Heroux and Brauer 1965 Intoccia and van Middlesworth 1959) indicate that the increased thyroidal turnover in rats induced by cold expo sure may be related to thyroxine depletion by an increased rate of fecal excretion In hamsters the changes observed in thyroid function upon cold exposure may be a response seen in hibernators in their adaptation to cold and in preparation for even tual hibernation as suggested by Knigge (1963)

The results of the present investigation are in contrast to the widely quoted conclusions of Brown Grant *et al* (1954 a) that the thyroid function in the rabbit is stimulated by cold It should be noted that in half of their cold exposed rabbits no stimulation of the thyroid was observed Thus in opposition to the rat and hamster, the rabbit does not appear to increase its thyroid function in the cold It may not be required since the metabolic response to cold is not directly dependent on increased activity of the thyroid (Hsieh and Carlson 1957 Sellers and You 1959), and since according to Cottle (1960) an augmented thyroidal secretion does not seem to be a prerequisite to development of the altered thermogenesis characteristic of cold acclimation In agreement with the present investigation the thyroidal retention curve of dogs have showed no changes whatsoever in response to acute and extended cold in a similar study of thyroid function (Wilson *et al* unpublished data)

In summary this study demonstrates the multicompartmental aspect of the thyroidal release of iodine, the marked interindividual variety of maximum uptake time to peak and release rate the intraindividual inconsistency in maximum uptake but reproducibility in time to peak and individual release rate and the failure of the thyroid to respond to cold exposure in the rabbit

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References

BERMAN M Iodine kinetics In *Methods in Investigative and Diagnostic Endocrinology* Vol I S A Berson Ed Elsevier Amsterdam 1972 pp 172—203

BERMAN M M F WEISS and E SHAHN Some formal approaches to the analysis of kinetic data in terms of linear compartmental systems *Biophys J* 1962 2 289—316

BERMAN M E HOFF M BARANDES D V BECKER M SONNENBERG R BENUA and D A KOUTRAS Iodine kinetics in man—a model *J clin Endocr* 1968 28 1—14

BOTTARI P M The concentration of thyrothrophic hormone in the blood of the rabbit under different experimental conditions *Ciba Found Colloq Endocr* 1957 11 52—69

BROWN GRANT K Changes in the thyroid activity of rats exposed to cold *J Physiol* (Lond) 1956 131 52—57

BROWN GRANT K The feed back hypothesis of the control of thyroid function *Ciba Found Colloq Endocr* 1957 10 97—120

BROWN GRANT K and J C GIBSON The metabolism of exogenous and endogenous thyroid hormone in the rabbit *J Physiol* (Lond) 1955 127 341—351

BROWN GRANT K and G PETHES The response of the thyroid gland of the guinea pig to stress *J Physiol* (Lond) 1960 151 40—50

BROWN GRANT K G W HARRIS and S REICHLIN The effect of emotional and physical stress on thyroid activity in the rabbit *J Physiol* (Lond) 1954 b 126 29—40

BROWN GRANT K C VON EULER G W HARRIS and S REICHLIN The measurement and experimental modification of thyroid activity in the rabbit *J Physiol* (Lond) 1954 a 126 1—28

COTTLE W H Role of thyroid secretion in cold acclimation *Fed Proc* 1960 19 Suppl 5 59—63

COTTLE M and L D CARLSON Turnover of thyroid hormone in cold exposed rats determined by radioactive iodine studies *Endocrinology* 1956 59 1—11

DANIEL P M M M GALE and O E PRATT Advantages of iodine 125 for studying thyroid function in experimental animals *Nature* (Lond) 1962 196 1065—1066

D ANGELO S A Adenohypophysial function in the guinea pig at low environmental temperature *Fed Proc* 1960 19 Suppl 5 51—56

FRENCH N R Iodine metabolism in wild jack rabbits In *Oklahoma Conference—Radioisotopes in Agriculture* USAEC Rept TID 7578 US Government Printing Office 1959 pp 113—121

FRENCH N R Comparison of radioisotope assimilation by granivorous and herbivorous mammals *Proc int Symp Stockholm 25—29 April 1966* Pergamon Press Oxford 1966 pp 665—673

GALTON V A and B C NISULA Thyroxine metabolism and thyroid function in the cold adapted rat *Endocrinology* 1969 *85* 79—86

GREER M A and H F SCHULL A quantitative study of the effect of thyrotropin upon the thyroidal secretion rate in euthyroid and thyrotoxic subjects *J clin Endocr* 1957 *17* 1030—1039

HARPER P V W D SIEMENS L A LATHROP and H ENDLICH Production and use of iodine 125 *J nucl Med* 1963 *4* 277—289

HEROUX O and R BRAUER Critical studies on determination of thyroid secretion rate in cold adapted animals *J appl Physiol* 1965 *20* 597—606

HSIEH A C L and L D CARLSON Role of the thyroid in metabolic response to low temperature *Amer J Physiol* 1957 *188* 40—44

INTERNATIONAL ATOMIC ENERGY AGENCY Consultants meeting on the calibration and standardisation of thyroid radioiodine uptake measurements November 28—30 1960 *Brit J Radiol* 1962 *35* 205—210

INTERNATIONAL COMMISSION ON RADIOLOGICAL PROTECTION *Recommendations of the International Commission on Radiological Protection as Amended 1959 and Revised 1962* ICRP Publication 6 Pergamon Press Oxford 1964

INTOCCIA A and L VAN MIDDLESWORTH Thyroxine excretion increase by cold exposure *Endocrinology* 1959 *64* 462—464

ITOH S T HIROSHIGE T KOSEKI and T NAKATSUGAWA Release of thyrotropin in relation to cold exposure *Fed Proc* 1966 *25* 1187—1192

KNIGGE K M Neuroendocrine mechanisms influencing ACTH and TSH secretion and their role in cold acclimation *Fed Proc* 1960 *19* Suppl 5 45—51

KNIGGE K M Thyroid function and plasma binding during cold exposure of the hamster *Fed Proc* 1963 *22* 755—760

KNIGGE K M R S GOODMAN and D H SOLOMON Role of pituitary adrenal and kidney in sereval thyroid responses of cold-exposed hamsters *Amer J Physiol* 1957 *189* 415—419

KNIGGE K M D H SOLOMON and S M BIERMAN Time relationships in the release of pituitary thyrotropin and thyroidal I[131] after acute exposure of the hamster to cold *XXI Int Cong Physiol Sci Buenos Aires 1959* Abstr Communications 1959 pp 146—147

KONNO N and T KOSEKI Changes in blood TSH levels in rats exposed to cold (in Japanese) *Folia endocrinol jap* 1967 *42* 1227—1231

MATTHEWS C M E Half life of iodine-125 *Phys Med Biol* 1960 *5* 45—47

MYERS W G and J C VANDERLEEDEN Radioiodine 125 *J nucl Med* 1960 *1* 149—164

PITT RIVERS R and J R TATA *The Thyroid Hormones* Pergamon Press London 1959 pp 32—33

PORATH M B A HOCHMAN and J GROSS A comparison of iodine-125 and iodine-131 as tracers in the diagnosis of thyroid distase I Physical aspects *J nucl Med* 1966a *7* 88—98

PORATH M B A HOCHMAN and J GROSS A comparison of iodine 125 and iodine 131 as tracers in the diagnosis of thyroid disease II Clinical aspects *J nucl Med* 1966b *7* 99—106

RALL J E J ROBBINS and C G LEWALLEN The thyroid In *The Hormones* Vol V (G Pincus K V Thimann and E B Astwood Eds) Academic Press New York and London 1964 pp 159—439

SELLERS E A and S S YOU Role of the thyroid in metabolic responses to a cold environment *Amer J Physiol* 1950 *163* 81—91

STEVENS C E S A D'ANGELO K E PASCHKIS A CANTAROW and F W SUNDERMAN The response of the pituitary thyroid system of the guinea pig to low environmental temperature *Endocrinology* 1955 *56* 143—156

STORAASLI J P S A ROSENBERG and H L FRIEDELL Effect of food and [illegible] on th[illegible] od uptake of radioiodine (I[131]) in rats *Proc Soc exp Biol* (N Y) 1953 *83* 148—150

STRAW J A and M J FREGLY Evaluation of thyroid and adrenal pituitary function during cold acclimation *J appl Physiol* 196[illegible] *23* 8[illegible]—8[illegible]0

TAUROG A J C PORTER and D T THIO Nature of th[illegible] [131]I [illegible] found r[illegible] ed into the thyroid veins of rabbits dogs and cats before and after TSH administration *Endocrinology* 1964 *74* 902—913

WERNER S C B C SEEGAL and E F OSSERMAN Immunologic and biologic characterization of antisera to beef thyrotropin preparations *J clin Invest* 1961 *40* 9[illegible]—10[illegible]

WOOD D H E E ELEFSON N G HORSTMAN and L K BUSTAD Thyroid uptake of radioiodine following various routes of administration *Hlth Phys* 1963 *9* [illegible]—1220

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Glycogen Depletion Pattern in Human Muscle Fibres During Distance Running

By

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Abstract

Costill D L , P D Gollnick E D Jansson B Saltin and E M Stein *Glycogen depletion pattern in human muscle fibres during distance running* Acta physiol scand 1973 *89* 374—383

Muscle biopsy and venous blood samples were taken from 9 subjects before and after a 30 km race Additional measurements were made when 8 subjects ran 20 5 km of the course 2—3 weeks later Information about total metabolism and the glycogen depletion pattern in the fibres of the thigh muscle during prolonged running has been obtained from these measurements The average oxygen uptake and respiratory exchange ratio were 4 7 l × min^{-1} (83 % of maximal oxygen uptake) and 0 90 respectively An average intramuscular tryglyceride reduction of 2 2 mmoles × kg^{-1} (30 %) accounted for only part of the lipid combusted Muscle glycogen declined 56 % (from 98 to 42 mmoles of glucose units × kg^{-1}) during the race Histochemical staining of the muscle for glycogen revealed a marked depletion in the slow twitch fibres Only a small glycogen depletion occurred in the fast twitch fibres This suggests a primary reliance upon slow twitch fibres during prolonged running The results also point to the limitations of assessing substrate utilization or availability in working fibres from muscle samples with mixed fibre populations

Complete glycogen depletion in the leg muscles of man is not usually seen after intense distance running (Costill *et al* 1971 a 1971 b and Karlsson Diamant and Saltin 1971) This was also true when running was performed on 3 consecutive days where muscle glycogen prior to exercise was progressively lower each day (Costill *et al* 1971 b) In contrast exhaustion during bicycle exercise coincides with depletion of muscle glycogen (Hermansen Hultman and Saltin 1967) Thus it appears that differences exist between prolonged running and bicycle exercise

Selective glycogen depletion in human muscle fibres has been found during bicycle exercise (Gollnick *et al* 1972 1973 a 1973 b) Slow twitch fibres are the first to be depleted of their glycogen at work intensities demanding 60—80 % of an individual's maximal oxygen uptake Fast twitch fibres also become glycogen depleted when exercise is continued to exhaustion In view of the apparent differences in glycogen depletion during running and cycling a study was undertaken to evaluate whether

or not a selective glycogen depletion occurs in the muscle fibres of the leg during running Venous blood samples and muscle biopsy samples from the thigh were also taken and analyzed to provide a rather complete picture of substrate mobilization and utilization in prolonged running

Subjects

Nine men with a fairly wide range of maximal oxygen uptake ($\dot{V}o$ max) were studied Their mean age was 27 (22—37) years and height and weight were 185 cm and 74 kg respectively All of the subjects were trained and accustomed to distance running but none of national caliber

Methods and procedures

Oxygen uptake ($\dot{V}o_2$) *was measured with the Douglas bag technique* The expired volume was measured in a Tissot spirometer or a calibrated gas meter (accuracy ± 1 5 %) Gas analyses were performed with the Haldane or Scholander technique Heart rates were determined by counting at least 20 QRS complexes from electrocardiogram (ECG) rcordings When running the course the ECG signals were recorded on magnetic tape and thereafter replayed (Svenska Radio AB Stockholm) The weight of the tape recorder was 0 35 kg Blood lactate (Scholz *et al* 1959) glucose (Hjelm and de Verdier 1963) free fatty acids (FFA) (Trout *et al* 1960) glycerol (Wieland 1957) and hematocrit were determined from blood taken from an ante cubital vein. The muscle samples, taken from the lateral portion of the quadriceps muscle with a needle (Bergstrom 1967) were carefully freed from blood connective tissue and fat and divided into 3 parts Two parts were weighed frozen in liquid nitrogen and stored at −80 C until subsequently analyzed for total glycogen (Karlsson Diamant and Saltin 1971) and tri glyceride content (Chernick 1969) Glycogen and triglyceride concentrations are expressed as wet weight values The water content of the muscles was 0 8 % lower after as compared to be fore running This small difference should not alter the basic findings of this study

The remaining part of the muscle sample was mounted onto a specimen holder in OCT em bedding medium (Ames Tissue Tek) and frozen in isopentane cooled to the temperature of liquid nitrogen Ten μ thick sections were cut in a cryostat at −20 C and mounted on cover glasses for histochemical analysis Myofibrillar adenosine triphosphatase activity was estimated with the method of Padykula and Herman (1955) and the fibres classified as slow twitch (ST) or fast twitch (FT) as previously described (Gollnick *et al* 1972) The distribution of glycogen in the fibres was estimated from serial sections (16 μ thick) stained with the period acid Schiff's (PAS) reaction (Pearse 1961) The staining intensity of each muscle fibre was subjectively rated as dark moderate light or negative as described previously (Gollnick *et al* 1972) All fibres are stained dark with the PAS reaction when the glycogen content of non exercised mu cle is above 80—100 mmoles of glucose units × kg^{-1} and it is not poss ble to discern differences in glycogen concentrations between fibres (Gollnick *et al* 1972 1973 a, 1973 b) The study was pe formed in connection with a 30 km ace (Lidingolopp t)

Since 2500 runners participated in the 30 km race it was only poss ble to obtain muscle and blood samples before and after the competition No measurements were performed during the race except for the careful notation of when the subjects passd 6 0 15 0 and 20 5 km on the course Blood samples were taken immediately after the race and the subjects then returned to the laboratory where muscle biopsies (30—60 min later) e taken Although some re synthesis of glycogen could ha e occurred n the muscle during the tim interval between termination of the race and the taking of the biopsy it was p obabl not enough to s gnificant ly alter the basic findings of this tudy (P hl 1973) Two to 3 weeks aft r the race 8 of the 9 subjects (one runner injured his back shortly after the race) ran the first 20 5 km of the course attempting to maintain the same pace as in the actual ace As shown in F g 1 the were very successful in this pacing For all subjects the time difference at any che k point did not exceed 2 min. On this bas s we felt justified to include the changes in blood metabolites from samples taken during the re un w th those obtained befor and after the actual com petition This was also true for the results of the muscle b opsy taken afte running 20 5 km

The race was run on good paths through the fo est and over f elds Th start and finish were at comparable elevation but the course included several mall hills with the total ele ation being approximately 425 meters (Fig 2) The mean work time in the rac was 147 min with the fastest and slowest subjects completing the 30 km in 1 3 and 171 m n respectively T

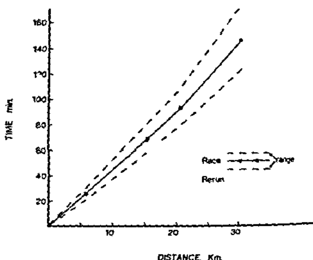


Fig. 1. Average time of the subjects at selected points for the race and rerun and finishing time

winning time was 101 min. As seen in Fig. 1, the pace during the first 20 km was slightly faster than that for the last 10 km.

All of the subjects consumed a carbohydrate enriched diet several days prior to the race and the rerun. During the run they were allowed to drink ad libitum. Most of them consumed between 0.5 and 1.0 l of a sugar solution resulting in the ingestion of 25—50 g of glucose.

The ECG while rerunning the course was recorded continuously and $\dot{V}_{O_2}$ determined 3 times. These later measurements were distributed along the course at points selected as being representative of the whole course. The first point started after 6.0 km and was over rather flat terrain. The 2nd and 3rd measurements were made after 15.0 (slightly downhill) and 20 (uphill) km respectively. The nose-clip and respiratory valve were given to the subjects while they were running. During the collection of the expired air the bag and connecting tube were carried by a person running along at the subject's pace. The subjects stopped for 20—40 s after each $\dot{V}_{O_2}$ measurement and blood samples were taken. After finishing 20.5 km the subjects returned to the laboratory where muscle biopsies were taken.

On a separate day the subjects also ran on a treadmill. A submaximal speed was chosen for each subject to correspond to his average speed during the race. This was done with the treadmill level. They also ran at a speed and grade sufficient to elicit $\dot{V}_{O_2}$ max. Heart rates and $\dot{V}_{O_2}$ measurements were made during the runs. Blood samples for lactate determination were taken after the completion of the treadmill runs.

Results

The $\dot{V}_{O_2}$ max of the subjects averaged 4.7 l/min or 65 ml/kg × min with a range from 56 to 76 ml/kg × min. Blood lactate after $\dot{V}_{O_2}$ max tests on the treadmill averaged 11.1 mmol × l^{-1} (range 9.2—15.4 mmol × l^{-1}). The runners utilized 71 (65—78 %) of their $\dot{V}_{O_2}$ max when running lightly dressed on the level treadmill at "race" speed (no wind resistance) (Table I). Post-exercise blood lactate after this exercise was 2.8 mmol × l^{-1}. Values ranging from 69 to 100 % of $\dot{V}_{O_2}$ max were observed during the rerun at race speeds. For each subject the lowest and highest $\dot{V}_{O_2}$ occurred during down- and uphill running respectively. The average $\dot{V}_{O_2}$ during the rerun was 3.86 l/min or 83 % of $\dot{V}_{O_2}$ max. The corresponding mean values for heart rate and respiratory exchange ratio (R value) were 171 b/min and 0.90 respectively (Table I). A more detailed illustration of the heart rate response for one representative subject is given in Fig. 2. It can be seen that the heart rate reached

TABLE I Oxygen consumption respiratory exchange ratio and heart rate for the treadmill tests and during running on the course

Condition	Oxygen uptake l × min⁻¹	Percent $\dot{V}_{O_2}$ max	Respiratory exchange ratio	Heart rate beats/min
Maximal treadmill	4 72	100	1 09	189 2
exercise	(3 84—5 64)		(1 05—1 20)	(170—20?)
Race pac	3 28	71	0 92	158 4
treadmill flat	(2 72—3 95)	(65—78)	(0 89—0 99)	(151—16?)
Rerun	3 8?	83	0 91	169 6 (172 8)*
flat	(3 04—4 65)	(75—97)	(0 87—0 98)	(159—177)
Rerun	3 59	76	0 88	1?0 6 (174 0)*
slightly downhill	(3 13—4 39)	(67—86)	(0 80—0 95)	(163—179)
Rerun	4 14	90	0 88	174 8 (180 0)*
uphill	(3 55—4 97)	(80—100)	(0 82—0 94)	(166—200)

Values within parentheses are the ranges Values within parenthesis and marked by a star ( )* are the mean heart rates at the time of the oxygen uptake measurements made when the subjects were running on the course

a level 20—30 b × min⁻¹ below the maximal rate rather quickly Thereafter a gradual increase occurred The heart rate approached maximal values for shorter intervals which coincided with the subjects running uphill

A marked reduction in muscle glycogen occurred in all subjects during the race (Table II) However after the race none of the subjects exhibited a muscle glycogen content lower than 23 mmoles of glucose units × kg⁻¹ The mean post race value was 42 mmoles of glucose units × kg⁻¹ The mean muscle glycogen depletion that occurred during the race was 56 mmoles of glucose units × kg⁻¹ After the rerun 50 mmoles of glucose units × kg⁻¹ of glycogen remained in the thigh muscle with the lowest value being 25 mmoles × kg⁻¹ (Table II)

The findings for glycogen depletion in the fibres are illustrated in Fig 3 Before the start of the race all FT and 94 % of the ST fibres were rated as PAS dark After

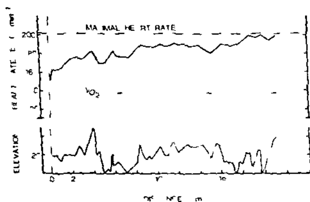


Fig H a t rate of one subject wh le r nn g 20 5 k n of th 30 km rse Fluctuat ons i art ate a e n to parallel hanges n d el ation of the ou s l wer po tions of th figure The points on th h e $\dot{V}_{O_2}$ measure n t w e made a e al o d t d

TABLE II Changes in muscle glycogen and triglyceride concentrations during prolonged running

Subject	Glycogen—mmoles of glucose unit × kg^{-1} 0 km	20 5 km	30 km	Triglycerides mmol × kg^{-1} 0 km	30 km
ML	125	—	72	8 1	6 1
IO	122	25	55	9 0	5 0
WJ	10,	71	60	8 5	4 ?
AM	71	42	34	6 8	6 4
BS	91	47	31	8 1	5 4
SB	124	CC	28	5 2	5 0
HB	80	—	23	6 8	3 3
UW	87	42	25	6 5	4 5
LS	77	53	50	5 4	4 9
Mean	98	50	42	7 1	4 9

Values are wet weight concentrations

20 5 km of running only 6 % of the ST fibres were still rated as dark for PAS staining and the remaining fibres were moderately (73 %) or lightly (26 %) stained At his point none of the ST fibres were PAS negative After the race 1 % of the ST fibres were PAS dark but 34 % were negative The rest of the ST fibres were rather evently distributed between being moderately or lightly stained The glycogen depletion of FT fibres contrasted markedly to that of ST fibres After 20 5 km 7 % of the FT fibres were PAS light and 26 % moderately stained All the remaining fibres were still PAS dark The glycogen depletion pattern for FT fibres in the muscles obtained after the race was essentially that observed after 20 5 km of running The 93 % decline in PAS dark stained ST fibres and 34 % increase in PAS negative fibres after 30 km of running was a highly significant change ($P < 0\ 01$) in the staining pattern A significant decline (67 %—$P < 0\ 01$) in PAS dark staining FT fibres also occurred during the running However the difference in the change

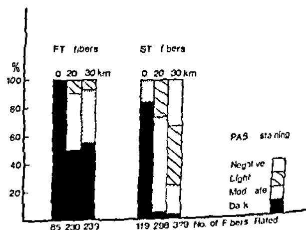


Fig 3 Summary of the PAS staining for glycogen in the FT and ST fibres at rest and after running 20 5 and 30 km The numbers below the bars represent the average number of fibres examined

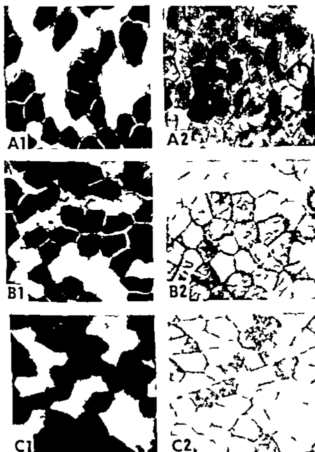


Fig 4 Photomicrographs (× 90) of the vastus lateralis muscle from one subject illustrating PAS staining intensity in the ST and FT fibres at rest (A series) and after running 20 5 (B series) and 30 (C series) km Micrographs A1 B1 and C1 are for myofibrillar adenosine triphosphatase activity showing the dark (FT) and light (ST) stained fibres A2 B2 and C2 are micrographs containing the PAS stained fibres Total glycogen for this subject was 87 42 and 25 mmol of glucose units × kg⁻¹ at rest, after running 20 5 km and after running 30 km respectively

in the staining pattern for ST and FT fibers was highly significant ($P < 0.01$) It should be mentioned that although the PAS staining has been evaluated with a 4 part system a continuum of staining intensities actually exists Fig 4 contains a set of photomicrographs showing the PAS staining pattern in the fibres in the muscle for one subject at rest after running 20 5 km and after completion of the race

The triglyceride concentration of the thigh muscle was 7 1 mmoles × kg⁻¹ before the start of work It was 2 2 (range 0 ?—4 0) mmol × kg⁻¹ lower after the race (Table II)

The changes in hematocrit and blood borne metabolites that occurred as a result of the prolonged running are illustrated in Fig 5 Some hemoconcentration did occur at the start of work but it was not large enough to account for the changes in glucose FFA glycerol or lactate concentrations Mean blood glucose increased from 3 5 to 5 mmol × l⁻¹ Lower values were observed later during the running but at the end of the race it was still 3 0 mmol × l⁻¹ Plasma FFA increased steadily to 1 3 mmol × l⁻¹ at the finish of the race Rather large individual variations in FFA concentrations

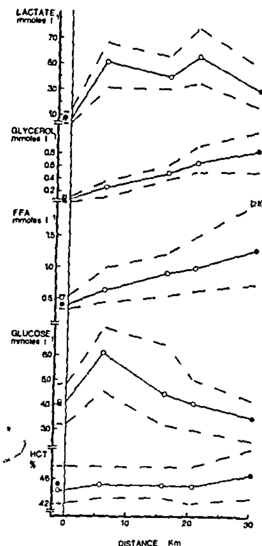


Fig 5

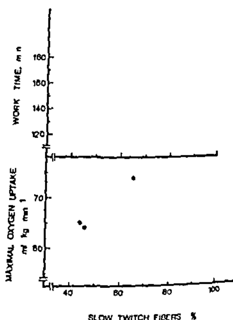


Fig 6

Fig 5 Changes in hematocrit blood glucose and lactate and plasma glycerol and free fatty acids during the 20.5 and 30 km runs The open circles are values from the 20.5 km run and the filled circles from 30 km run The broken lines above and below the solid lines represent the highest and lowest values respectively

Fig 6 Relationship between the percentage of ST fibres in the thigh and work time (upper panel) and ST fibres and $\dot{V}_{O_2}$ max (lower panel)

were observed both after the actual race and the rerun However of some interest was the finding that the subject with the highest plasma FFA level after the race also had the highest value in the rerun This subject also had the smallest glycogen depletion and the lowest R value The gradual increase in the glycerol concentration starting at 0.04 and reaching 0.8 mmol × l⁻¹ after the race is an indication of the

enhanced lipid mobilization that occurred during the prolonged work

Blood lactate concentration varied inconsistently during the run In prolonged work blood lactate concentrations usually reach a peak 10—30 min after the start of work (Bang 1936) Thereafter it either levels off or a reduction is observed with resting concentrations being gradually approached The explanation for the rather high values (5 7 mmol × l^{-1}) seen as late as 20 5 km of running in the present study most likely is the increased work intensity occurring while running on the hilly course At the end of the race the blood lactate was 3 mmol × l^{-1} This was also anticipated since the pace especially running uphill was somewhat lower for most of the runners over the last 8—9 km

Discussion

The most important finding from this study of prolonged running was the selective and marked glycogen depletion from the ST fibres with only a very minor reduction occurring in the FT fibres During prolonged cycling of equal intensity the ST fibres are also the first to become depleted of their glycogen (Gollnick *et al* 1972 1973 a) In cycling however the FT fibres also become depleted of their glycogen stores to a marked extent after 2—3 hours of work (Gollnick *et al* 1973 a)

The question then arises as to whether in running a different muscle fibre recruitment pattern exists or that the load on the thigh muscles is less as compared to bicycling If the latter explanation is correct the observation that some FT fibres were partially depleted of their glycogen after 80—100 min of running (20 5 km) may be explained by the increased load on this muscle during uphill running The lack of any further glycogen depletion in the FT fibres during the last 9 5 km of the race may have resulted from the fact that the subjects could not run but had to walk or jog up the steepest hills over the last part of the course The slower pace of the last portion of the race would support this contention

It has been suggested (Costill *et al* 1971 a 1971 b) that an empty glycogen store cannot explain the fatigue or exhaustion that occurs during distance running since considerable glycogen remains in the muscle after prolonged running The results of this study also show that considerable glycogen remained in the muscle when the subjects were near exhaustion However they also demonstrate that the picture is more complex than simply a direct relationship between total muscle glycogen and ability to perform long distance running The runners could not keep up their pace near the end of the race Two runners also experienced light cramps in their legs This happened in spite of the fact that the FT fibres which are recruited during very heavy work (Gollnick *et al* 1973 b) were still well filled with glycogen The reasons for the inability to recruit the FT fibres in this situation are obscure The exhaustion observed during the later part of the race most likely is related to the extensive use of the ST fibres which were depleted of their glycogen store From the results of this study it cannot be stated whether the glycogen depletion in the ST fibres or some other concomitantly occurring alteration in these fibres was the true cause of

fatigue However, these results and those from previous studies (Gollnick *et al* 1972 1973 a, 1973 b), do indicate that the determination of total muscle glycogen in a muscle sample where a selective depletion in some fibres has occurred does not reflect the glycogen consumption or the metabolic reserves of the working fibres

The energy expenditure for running the 30 km was approximately 2600 kcal (1 kcal = 4 19 kJoule) or 35 kcal per kg b wt These values have been arrived at by using the average V_{O_2} and R values obtained during the second run of the course (Table I) and extrapolating them to the average time for running the race Based on the R values approximately 35—40 % of the calories are derived from fat and 60—65 % from carbohydrates This means that 100—110 g of FFA should have been oxidized In skiing a marked reduction in the intramuscular triglycerides has been reported (Froberg and Mossfeldt 1971) The present results suggest that the lipid *content of the lateral part of the quadriceps muscle is also lowered by running* However the major contributor of lipid substrate during distance running seems to be extra muscular sources The high plasma FFA and glycerol levels of this study point to an enhanced lipolysis in adipose tissue

A glycogen depletion of 56 mmoles of glucose units$\times$kg^{-1} agrees with the observation in a previous study over the same distance (Karlsson and Saltin 1971) In that investigation the initial muscle glycogen content was also about 100 mmoles of glucose units$\times$kg^{-1} The calculated value for carbohydrate utilization for running the 30 km was approximately 350—400 g Of this 75—125 g could have come from extra muscular sources (liver and oral intake) The remaining 250 g must have been supplied by the intramuscular depots The mean glycogen reduction in the thigh was approximately 10 g$\times$kg^{-1} According to data from Costill *et al* (1971 a 1971 b) the glycogen depletion during running is about the same in the gastrocnemius muscle and only slightly higher in the soleus muscle Our data then indicate that a very large portion of man s muscle mass is engaged in running However the present study cannot provide any detailed information on this subject or on the relative rate of glycogen depletion in different muscle groups

The time difference between the first and the last subject was close to 1 hour In several studies with a wide range in performance a good correction has been demonstrated between maximal aerobic power and distance running (Costill 1967 and Karlsson and Saltin 1971) This was also the case in this study Moreover the work time for a runner with a certain V_{O_2} max in the present study was directly comparable to what was found 4 years ago when 10 other subjects ran the same distance (Karlsson and Saltin 1971) The percentage of ST fibres in the thigh of the present subjects was closely correlated to their V_{O_2} max Thus in this group of rather homogenously trained subjects an inverse relationship existed between ST fibres and work time These relationships illustrated in Fig 6 should not be expected to exist in a larger sample of untrained and trained subjects

In prolonged exercise there is a preferential recruitment of muscle fibres that now has also been demonstrated in running How human muscle fibres might be selectively recruited during work has been discussed earlier (Gollnick *et al* 1972 1973 a)

This selective recruitment theory is based on the observation that motor units are composed of the same fibre types that are innervated by motoneurons with different thresholds (Henneman and Olson 1965) An important consequence of the selective use of muscle fibres which was discussed briefly above is that exercise induced changes in the cellular constituents may occur only in one fibre type Thus estimation of muscular components such as glycogen phosphagens lipids or electrolytes from mixed muscle samples are probably underestimates of the true changes that occur in the active fibre

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References

BANG O The lactate content of the blood during and after muscular exercise in man. *Skand Arch Physiol* 1936 *74* Suppl 10 51—82

BERGSTROM J Muscle electrolytes in man. *Scand J clin Lab Invest* 1962 Suppl 68

CHERNICK S S Determination of glycerol and acylglycerol. *Methods in Enzymology* 1969 *14* 627—630

COSTILL D L The relationship between selected physiological variables and distance running performance *J Sports Med* 1967 *7* 61—66

COSTILL D L Metabolic responses during distance running *J appl Physiol* 1970 *28* 251—255

COSTILL D L, K SPARKS R. GREGOR and C TURNER Muscle glycogen utilization during exhaustive running *J appl Physiol* 1971 a *31* 353—356

COSTILL D L R. BOWERS G BRANAM and K. SPARKS Muscle glycogen utilization during prolonged exercise on successive days *J appl Physiol* 1971 b *31* 834—838

FROBERG S O and F MOSSFELDT Effect of prolonged strenuous exercise on the concentration of triglycerides phospholipides and glycogen in muscle of man *Acta physiol scand* 1971 *82* 167—171

GOLLNICK P D K. PIEHL C W SAUBERT IV R B ARMSTRONG and B SALTIN Diet exercise and glycogen changes in human muscle fibers *J appl Physiol* 1972 *33* 421—425

GOLLNICK P D R. B ARMSTRONG C W SAUBERT IV W L SEMBROWICH R. E SHEPHERD and B SALTIN Glycogen depletion patterns in human skeletal muscle fibers during prolonged work *Pflugers Archiv ges Physiol* 1973 a. In press

GOLLNICK P D R B ARMSTRONG W L SEMBROWICH R. E SHEPHERD and B SALTIN Glycogen depletion pattern in human skeletal muscle fibers after heavy exercise *J appl Physiol* 1973 b In press

HENNEMAN E and C B OLSON Relationship between structure and function in the design of skeletal muscle *J Neurophysiol* 1965 *28* 581—598

HERMANSEN L E HULTMAN and B SALTIN Muscle glycogen during prolonged severe exercise *Acta physiol scand* 1967 *71* 129—139

HJELM M and C H DE VERDIER A methodological study of the enzymatic determination of glucose in blood *Scand J clin Lab Invest* 1963 *15* 415—428

KARLSSON J B DIAMANT and B SALTIN Muscle metabolites during submaximal and maximal exercise in man *Scand J clin Lab Invest* 1971 *26* 385—394

KARLSSON J and B SALTIN Diet, muscle glycogen and endurance performance *J appl Physiol* 1971 *31* 203—206

PADYKULA M A and E HERMAN The specificity of the histochemical method of adenosine triphosphatase *J Histochem Cytochem* 1955 *3* 170—195

PEARSE A G E *Histochemistry—Theoretical and Applied* Boston Little Brown 1961 Appendex 9 p 832

PIEHL K Time course for glycogen storage in muscle fibers of man following exercise induced depletion *Acta physiol scand* 1974 *90* In press

SCHOLZ R H SCHMITZ T BUECHER and J O LAMPER. Über die Wirkung von Nystatin auf Bäckerhefe *Biochem Z* 1959 *331* 71—86

TROUT D L E H ESTES JR. and S J FRIEDBERG Titration of free fatty acids of plasma A study of current methods and a new modification. *J Lipid Res* 1960 *1* 199—202

WIELAND O Eine enzymatische Method zur Bestimmung von Glycerin *Biochem Z* 1957 *329*, 313—317

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Analysis of Threshold Currents during Microstimulation of Fibres in the Spinal Cord

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Abstract

W J ROBERTS and D O SMITH *Analysis of threshold currents during micro stimulation of fibres in the spinal cord* Acta physiol scand 1973 89 384—394

Fibres within the dorsal spinocerebellar tract of the cat were stimulated with current pulses from a microelectrode With precise tracking single nodes of Ranvier of a fibre could be localized and the current required for activation studied as a function of the electrode position The threshold stimulus amplitude was found to increase nonlinearly with distance from the node The rate of increase in threshold was found to be less pronounced when the position of the electrode tip was changed axially rather than transversely to the node The rates of increase with distance tended to decrease as the estimated fibre diameter became larger

Since it was introduced by Wall *et al* (1956) microstimulation has been used to an increasing extent for selective activation of specific fibre populations and/or single cells within the central nervous system The threshold currents during micro stimulation in the spinal cord have been examined for the axons of single Ia in hibitory interneurons (Jankowska and Roberts 1972) and Renshaw cells (Jankowska and Smith 1973) However these analyses were complicated by the possibility of spike generation at more than one site on the investigated fibres such as adjacent nodes of Ranvier or nearby axon collaterals

The present study was designed to analyze without these complications the relationship between the amplitude of the stimulus current applied through a micro electrode and the distance from which fibres in the dorsal spinocerebellar tract can be activated in the cat spinal cord It was found that single nodes of Ranvier of a given fiber could be precisely localized and the current required for activation were measured for many sites at known distances from each node This informa

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tion can be useful during applications of microstimulation such as stimulation of specific fiber tracts within the central nervous system or localization of the axon of a single cell

Methods

Under ether anesthesia the left sciatic hamstring and quadriceps nerves were cut in the cat s hindlimb and mounted for stimulation The quadriceps nerve was mounted on an electrode sewn under the skin while the other nerves were stimulated in a paraffin pool. The spinal cord was exposed by a laminectomy from L1 to L5 and from Th10 to Th12 Care was taken to prevent obstruction of the major dorsal veins of the cord after opening the dura. The dorsal columns and the contralateral spinal half were sectioned at Th11 in order to simplify the identificat on of DSCT cells After surgery the cats were anesthetized with alpha chloralose (40—60 mg/kg) and paralyzed with Flaxedil The cats temperature blood pressure and ex pired CO_2 were monitored and regulated by radiant heat intravenous infusion of Dextran and artifi ial respiration rate respectively

Two microelectrodes were inserted into the cord as illustrated in Fig 1 A Both were filled with NaCl and broken to tip diameters of 1 5—2 5 μm They could be moved independ ntly through the use of 2 motor-driven micromanipulators (Eide and Kallstrom 1968) Recording and stimulating through the microelectrodes was accomplished with two solid state amplifiers Conventional techniques were employed in stimulating and recording from the peripheral nerves and the spinal cord surface

Action potentials of single cells located in Clarke s column at the L3—L4 level were recorded extracellularly with one microelectrode Only cells monosynaptically excited from hindlimb Ia afferents and antidromically activated from the ipsilateral dorsolateral funiculus at Th10 were studied Such cells were shown to project to the cerebellum as part of the dorsal spinocerebellar tract (Lundberg and Oscarsson 1960) The axons of these cells were activated by weak rectangular current pulses through the second mic oelectrode (Fig 1 A cf Jankowska and Roberts 1972) The threshold for activation of a gi en cell was measured as the el ctrode penetrated the cord at L1—L2 in regular increments in the vertical plane rather than during withdrawal to avoid any low resistance pathways created by fluid filling the vacant electrode track Careful cleaning of the cord surface at the site of entry of the electrode was used to prevent compression

Typical recordings f om which all data were taken are shown in Fig 1 B and C The lower trace of Fig 1 B an extracellular record from a cell in Clarke s column illustrates the action

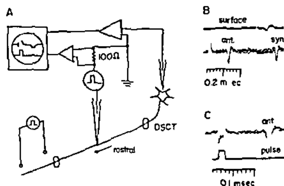


Fig 1 Experimental arrangement The schematic diagram in A shows the surface stimulat on electrodes at the left used to activate antidromically fibers in the dorsal spino-cerebellar tract (DSCT) at Th 10 The spikes were recorded extracellularly in Clarke s column in the lumbar enlargement as indicated at the right The stimulating microelectrode n the center was mo ed systematically to d fferent sites (dashed figures) The rec ording circuitry is illustrated Typical recordings are shown in B and C In B the upper record was obtained from the cord dorsum at L4 Th artifact asso ated with periph e al nerve stimulation precedes the incom ng volley The lower trace shows uperimposed antidromic responses of a single Clarke s column cell to microelectrode st mulation in the DSCT (ant) followed by synaptically e oked spik s (syn) In C the antidromi response shown on an expanded tim scale (upper record) together with a record of the stimulus current.

potentials evoked antidromically from the DSCT and monosynaptically following peripheral nerve stimulation The upper trace shows the concomitant potentials recorded from the cord dorsum at L4 In Fig 1 C the antidromic spike is presented on an expanded time scale to allow more precise latency measurements The second trace indicates the stimulus pulse amplitude The stimulus current was measured as the potential drop across a 100 ohm resistor placed between the stimulating electrode and the silver reference electrode inserted in the muscles of the back (cf Fig 1 A)

A collision test between impulses evoked synaptically by peripheral nerve stimulation and in response to the microelectrode pulse was used for most cells studied to ascertain that the latter were due to antidromic activation (cf Jankowska and Roberts 1972) The conduction velocity of the investigated fiber was determined by measuring the latency differences at the soma for spikes evoked by barely suprathreshold pulses applied through the stimulating microelectrode at the level of Th12—L1 and through a bipolar surface electrode located on the dorsolateral funiculus at Th10 (Fig 1 A)

Positioning accuracy of the microelectrode was ±2 μm along the electrode axis and less than ±10 μm both rostrocaudally and mediolaterally Assuming no bending of the electrode the uncertainty of the position of the electrode shaft is estimated to be less than about 18 μ

Results

1 *Identification of Single Nodes of Ranvier*

The axons of single cells in the dorsal spinocerebellar tract were first located approximately by means of antidromic stimulation with cathodal current pulses of 15 μA passed through the stimulating microelectrode (cf Jankowska and Roberts 1972 Jankowska and Smith 1973) A number of closely spaced tracks were then used to find sites requiring less current for activation No attempt was made to find the minimum current required however sufficient tracking was done to activate all 14 cells studied with 0 1 ms cathodal pulses of 0 3 to 3 0 μA

Curves relating the threshold current and the depth of the stimulating electrode were prepared for a number of tracks around each axon Those obtained from 2 separate regions about one fiber each region characterized by low thresholds are plotted in Fig 2 B and C The data points obtained at 50 or 25 μm increments in depth along each track are interconnected and the track numbers are listed at the right in each figure in the order of decreasing minimum threshold The locations of the tracks used are mapped in Fig 2 A on a horizontal coordinate plane through the spinal cord The indicated positions of the axon (wide line) and the nodes along the axon (gaps in line) were inferred from the threshold measurements as described below

The low minimum in the threshold current recorded in track 18 (Fig 2 C) suggested that a node was located nearer to it than to the neighboring tracks (Tasaki 1964) all of which had higher thresholds The location of a second node is indicated by the minimum values obtained from tracks 10 11 26 and 27 (Fig 2 B) all of which seemed to be nearly equidistant from the node The minimum threshold in track 12 was 7 9 μA sufficiently high to exclude the presence of a third node between the two shown The threshold currents generally changed quite smoothly with depth and the minimum thresholds were found at approximately the same depth below the surface of the cord for each node The minimum threshold values also increased progressively for tracks at longer distances (rostrocaudally or medio-

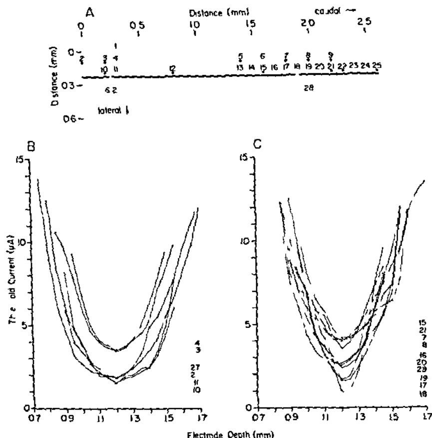


Fig 2 Threshold current for antidromic activation as a function of stimulating electrode depth below the cord surface In A a map of the tracks from which the cell could be activated antidromically by 0 1 ms cathodal pulses is shown The tracks (black circles) are numbered arbitrarily The heavy black line represents the estimated axon location and the two gaps in the line represent the estimated node locations. The cell body is located about 6 4 cm caudally to track number 18 In B and C the threshold currents are plotted as a function of electrode depths below the cord surface for the tracks in the regions of the two nodes shown in A Data points for a given track are connected by solid lines and their numbers corresponding to those in A are listed on the right in the order of decreasing minimum threshold values

laterally) from the region of lowest values Our observation that only one low threshold focus was found in any transverse plane indicated that these axons do not branch at this level (L1—L2) We presumed that each axon traveled in a straight line from caudal to rostral an assumption supported by the measured nodal positions whenever 2 or 3 nodes were localized along a single fiber The measured internodal distances were also found to be consistent with the conduction velocities of these fibers (Hursh 1939) The consistency of these observations strongly indicates that the low threshold sites correspond to regions close to single nodes

Tissue damage created by multiple electrode penetrations at small distances might

be expected to influence current measurements in nearby tracks A current hurt into a region of damaged tissue might reduce the rate at which the threshold current changes with depth No clear broadening of the curves is seen in tracks 10 11 ?6 and 27 (Fig 2 B) in which measurements were taken consecutively within a period of about twenty minutes However changes in the relationship of the threshold currents with depth were found when measurements were taken in a given track for a second time or in neighboring tracks two or more hours apart These changes probably resulted from tissue damage

The curves in Fig 2 C are slightly steeper than those in Fig 2 B The fact that all tracks around the first nodal region (Fig 2 B) were uniformly broader than those around the second (Fig 2 C) suggests that local variations in the nodal geometry (Gasser and Grundfest 1939) or in the local tissue properties can play a significant role in the effective current distribution

The systematic tracking performed in 100 μm increments rostrocaudally and mediolaterally in the horizontal plane (cf Fig 2 A) and in 50 μ increments along the depth of the electrode in the vertical plane provided a set of threshold coordinates from which a more precise estimate of node location could be determined by interpolation (see below) without damaging the fiber by penetration Only those tracks with low minimum thresholds (< 2 μA) at the same depth and adjacent to the track from which the lowest threshold was found were used for these estimates

2 *Relationship between threshold current and the distance of the stimulating electrode tip from the activated node of Ranvier*

In several cases the threshold current versus depth curve for a single track was steep enough at a uniquely low minimum threshold value to suggest that the electrode passed very close (< 30 μm) to the node (*e g* track 18 *in Fig* 2) The shapes of the threshold depth curves in these cases and in similar plots of Jankowska and Roberts (1972) for Ia inhibitory interneurones (in which minimum thresholds as low as 0 1 μA were reported) and of Jankowska and Smith (1973) for Renshaw cells (minimum thresholds as low as 0 2 μA) suggest that the threshold current strength is approximately proportional to the square of the distance between the node and the electrode for very short distances (< 150 μm)

Assuming such a relationship between threshold current and distance the position of each node relative to the electrode coordinates was estimated by interpolation using the threshold measurements obtained in the 3 or 4 tracks spaced at 100 μm intervals which gave the lowest threshold values about each node With this method the uncertainty associated with the estimates was less than 30 μm when the tracking was most complete such as for the more rostral node in Fig 2 A in which four suitable tracks could be used and less than 50 μm in the most imprecise case in which only 3 tracks passed within 120 μm of the estimated node position These uncertainties apply to the data to be presented in Fig 3—5 (see below) in which the

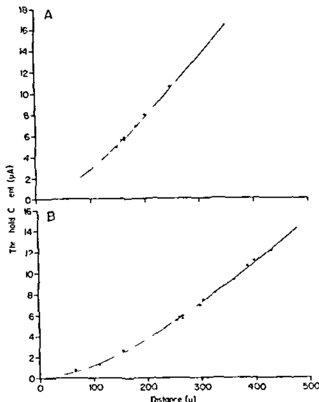


Fig 3 Threshold currents for antidromic activation from "transverse" sites as a function of the distance from the activated node The data in A (5 tracks 47 stimulation sites) and B (6 tracks 73 stimulation sites) are from two nodes of different fibers with conduction velocities measured to be 60 and 67 m/s respectively All data were obtained from tracks in which the electrode was in a plane approximately transverse to and intersecting the node The lines passing through the data were determined by iterative nonlinear regression techniques

distances plotted are the best estimates obtained with the above method The absolute threshold at any single stimulus site is a less accurate indicator of distance than that obtained by interpolation from several sites as other factors such as fibre diameter influence the current required for activation

After the node location had been estimated relative to the electrode coordinates the distance of the electrode tip from the node could be determined for all stimulus sites by simple triangulation procedures from the track locations and the electrode depths all of which were measured from the same reference coordinates The threshold currents for activation were then examined as a function of the distance between the stimulating electrode tip and the node at which the spike was generated Data were prepared for the eight nodes whose positions could be estimated with the most certainty and were then divided into two groups those obtained from stimulus sites located in a plane normal to the fibres and intersecting the nodes (e g threshold measurements from different depths in track 18 of Fig 2 A) which will be called transverse data and those from stimulus sites located axial to the nodes along the fibers (e g minimum threshold values from tracks 14—17 of Fig 2 A) which will be called axial data

Transverse data are illustrated for two node from two fibres of different con

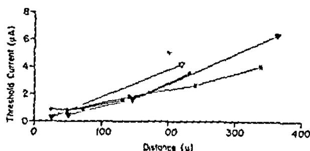


Fig 4 Threshold currents for antidromic activation from axial sites as a function of the distance from the activated node The data represent the minimum threshold currents from tracks which passed near (< 50 μm) to the internodal regions of 5 fibers whose conduction velocities ranged from 52—85 m/s The indicated distances were those from the electrode tip to the nearest node on each fiber The dashed line represents data from the same node as in Fig 3 A

duction velocities in Fig 3 A (60 m/s) and B (67 m/s) The thresholds appeared to increase nearly linearly for distances at least 20 times the fibre diameter (which was determined by dividing the measured conduction velocity by 6 Hursh 1939) However, as the electrode approached the node the relationship became curvilinear In fact if linearity of the data over the entire range of distances is assumed, least square lines which were fit to the data nearly always predicted thresholds significantly ($p < 0.01$) less than zero for distances less than 50 μm—a physiological impossibility This contradiction alone is sufficient to reject the hypothesis of linearity over the entire range of distances when the current source was in a transverse plane

Data obtained from axial sites within 50 μm of the fiber for 5 different nodes are shown in Fig 4 in which data from individual nodes are connected by lines the dashed line represents data from the same node as in Fig 3 A As in the transverse data the relationship between threshold and distance showed some nonlinearity mainly at short distances The rates at which the threshold currents changed with distance were smaller for sites axial to the nodes than for those normal to the same node For example the curve derived from normal data in Fig 3 A rises more steeply than that from the data axial to the same node represented by the dashed line in Fig 4 The same trend can also be seen in the data of Fig 2 C in which the minimum thresholds for tracks 15 and 21 located about 300 μm from the node in an axial direction were found to be nearly the same as for tracks 7 and 8 located about half that distance from the node but not so near to the myelinated parts of that fibre

In agreement with a suggestion by Jankowska and Roberts (1972) the slopes of the threshold distance curves had a tendency to vary inversely with the estimated fiber diameter for short distances (< 200 μm) To examine this relationship more carefully a curve was empirically fit to each set of data using an iterative regression procedure (Williams 1959) Two examples are plotted through the data in Fig 3 A and B The same procedure was used for data from three other fibers and the resulting curves are plotted together in Fig 5 A nonlinearity was found at short distances in each case but there was considerable variability in the relationship between absolute threshold and conduction velocity at these distances (< 200 μm) For distances greater than 200 μm where the threshold distance relation is essen

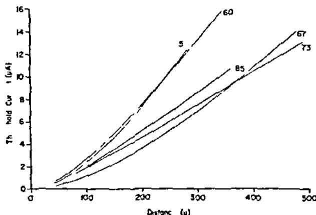


Fig 5 Threshold currents for antidromic activation from transverse sites as a function of the distance from the nearest nodes of five fibers The curves were obtained by regression techniques and are used to represent data similar to those in Fig 3 Each curve is based on more than 40 data points The conduction velocities (m/s) are shown for each fiber represented

tially linear the slopes of the threshold distance curves are inversely related to the conduction velocities of the 5 fibers the correlation coefficient being −0 83 This is a fairly significant correlation ($p < 0\,1$) BeMent and Ranck (1969 a) reported a similar relationship for larger distances (> 600 µm)

3 *Supra threshold block*

Microstimulation of a nearby node with supra threshold cathodal currents would be expected to produce a hyperpolarization of the adjacent nodes and possibly anodal block of the fiber This was tested in 10 cases in which the antidromic invasion failed to occur at 8 3 ± 2 7 (S D) times the threshold current strength for 0 1 ms pulses These values are similar to those reported by Jankowska and Roberts (1972) for Ia inhibitory interneurones but greater than those found by Katz and Miledi (1965) for unmyelinated axon terminals

After most of these supra threshold tests the threshold current was increased for up to several minutes even though the block occurred with currents less than 18 µA (because of current passing limitations imposed by the glass microelectrodes) These results indicate that possible fiber damage or changes in tissue structure or ionic distribution may result from these relatively strong currents passed through electrodes located quite close to the nodes (*cf* Stampfli 1958)

Discussion

The results of this study provide the first opportunity to analyze quantitatively the currents required to activate single nodes of Ranvier within the central nervous system of mammals They support the contentions of Jankowska and Roberts (1972) and Jankowska and Smith (1973) that the microstimulation techniques introduced by Wall *et al* (1956) are suitable for precise localization of fibers in the spinal cord In those studies however single nodes were not localized except for certain

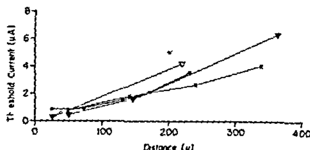


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The inverse relationship between the slopes of the threshold distance curves and the fiber diameter indicated by the observations of Jankowska and Roberts (1972) is also suggested by the present data For distances longer than 200 μm where linearity was assumed the correlation coefficient, r between the slope determined by least squares and the conduction velocity of the fibers for the five nodes represented in Fig 5 was -0.83 ($p < 0.1$) This indicates that nearly 70 % ($r^2 = 0.69$) of the variation in the slopes can be attributed to differences in fiber diameter

An inverse relation between the fiber diameter and the threshold distance slope was found by BeMent and Ranck (1969 a b) at large distances (> 600 μm) At these longer distances the tissue potential at nodes other than the nearest node may be changed significantly by the stimulus especially in the case of smaller fibers with shorter internodal lengths This could reduce the net depolarizing current passing through the nearest node and thereby increase the threshold of the small fibers relative to the larger ones This explanation for the different slopes however may not be adequate at shorter distances (< 500 μm) A theoretical analysis is needed to determine causality

The curvilinear relationship between threshold and distance seen in the data indicates that the stimulus effectiveness is not simply proportional to the tissue potential produced at the nearest node by the stimulus If it were the threshold should increase linearly with distance since the potential produced by a small current source in a volume conductor varies inversely with the distance except very close to the electrode (Rush 1962) A quantitative analysis of the relationship reported in this study is beyond the scope of this paper but will be dealt with in a subsequent publication (W J Roberts and D O Smith in preparation)

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References

BeMent S L and J B Ranck A quantitative study of electrical stimulation of central myelinated fibers *Exp Neurol* 1969 a *24* 147—170

BeMent S L and J B Ranck A model for electrical stimulation of central myelinated fibers with monopolar electrodes *Exp Neurol* 1969 b *24* 171—186

Eide E and Y Kallstrom Remotely controlled micromanipulator for neurophysiological use *Acta physiol scand* 1968 *73* 2A

Gasser H and H Grundfest Axon diameters in relation to the spike dimensions and the conduction velocity in mammalian A fibers *Am J Physiol* 1939 *127* 393—414

Hursh J B Conduction velocity and diameter of nerve fibers *Am J Physiol* 1939 *127* 131—139

Jankowska E and W J Roberts An [illegible] demonstration of the axonal projections of single interneurons in the cat *J Physiol* (Lond) 1972 *222* 597—622

Jankowska E and D O Smith Antidromic activation of Renshaw cells and the distribution of their axonal branches *Acta physiol scand* 1973 *88* 198—214

Katz B and R Miledi Propagation of electric activity in motor nerve terminals *Proc roy Soc B* 1965 *161* 453—482

Lundberg A and O Oscarsson [illegible] organization of the dorsal spino-cerebellar tract in the cat VII Identification of units by antidromic [illegible] from the cerebellar

cortex with recognition of five functional subdivisions. *Acta physiol. scand* 1960 *50* 3??–3?4
NICHOLSON P W., Specific impedance of cerebral white matter *Exp Neurol* 1965 *13* 3??–401
RANCK, J B and S L. BEMENT The specific impedance of the dorsal columns of cat An anisotropic medium. *Exp Neurol.* 1965 *11* 451—463
RUSH S., Methods of measuring the resistivities of anisotropic conducting media in situ. *J res nat Bur Stand* 1962 *C 66* 217—222.
STÄMPFLI, R., Reversible electrical breakdown of the excitable membrane of a Ranvier node. *An Acad bras. Ciencias* 1958 *30* 57—63
TASAKI I., A new measurement of action currents developed by single nodes of Ranvier *J Neurophysiol* 1964 *27* 1199—1206
WALL, P D W S MC CULLOCH J Y LETTVIN and W H PITTS The terminal arborization of the cat's pyramidal tract determined by a new technique *Yale J Biol Med* 1956 *?* 457—464
WILLIAMS, E., *Regression Analysis* New York. Wiley 1959

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The Influence of Mechanical Factors on the Response Characteristics of the Frog Muscle Spindle

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Abstract

HUSMARK I *The influence of mechanical factors on the response characteristics of the frog muscle spindle* Acta physiol scand 1973 89 395—405

The impulse response of the muscle spindle *in situ* was related to lengthening and tension of the whole muscle during stretch The results were compared with the impulse response as related to lengthening and tension obtained from stretches of the isolated encapsulated sensory region (isolated spindle) The aim was to find out if the force transmitting structures distorted the stretch stimulus and also to see if the adaptation of the spindle *in situ* was causally related to the extrafusal tension changes The results indicate that during stretch the extra and intra fusal fibres develop tension changes which have the same general characteristics and which are related to the impulse response in a regular way In some spindles the impulse response did not reflect the general tension of the muscle This fact lends support to the hypothesis that most of the mechanical deformation of the sensory endings as revealed by the impulse responses is produced by the lengthening of the intrafusal fibres Furthermore only a small part of the adaptive decline of the response can be ascribed to the extrafusal tissue tension

As a result of previous studies much knowledge has been gained about the functional properties of the frog muscle spindle *in situ* (Adrian and Zotterman 1926 Bronk 1929 B H C Matthews 1931 Katz 1950 Brown 1971 Kirkwood 1972) about the semi isolated spindle (Ito 1968 Jahn 1968) and about the isolated spindle (*cf* Katz 1950 Ottoson and Shepherd 1971)

In the present paper an attempt has been made to compare the response of a single spindle *in situ* and the tension development of the whole muscle with the response and the tension of the isolated spindle The experimental procedure was the same for both preparations and the parameters of stretch whenever possible were kept the same In this manner the experiment on the normal spindle *in situ* served as a control for the experiments on the isolated spindle and vice versa (*cf* Kirkwood 1972)

The tension change may be taken as a measure of the viscoelastic rearrangements occurring within the structure under stretch and by measuring the tension of the whole muscle it is possible to get information of some of the mechanical events in

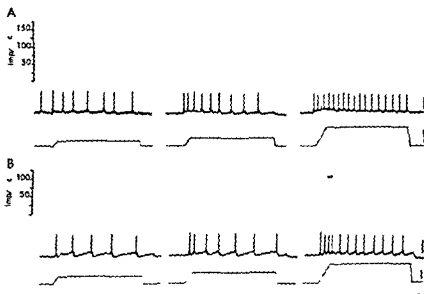


Fig 2 Impulse response and frequency of spindle in situ (A) and of isolated spindle (B) during stretches to increasing levels Upper vertical bar in A 0 1 mV in B 0 2 mV Lower vertical bar in A 2 mm in B 100 μm Length of preparation in A 20 mm in B 650 μm Time bar 100 ms

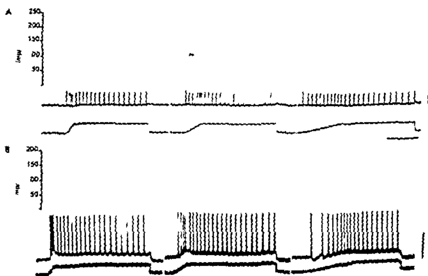


Fig 3 Impulse response and frequency of spindle in situ (A) and of isolated spindle (B) during stretches of decreasing velocities. Upper vertical bar in A 0 5 mV in B 2 mV Time bar 100 ms

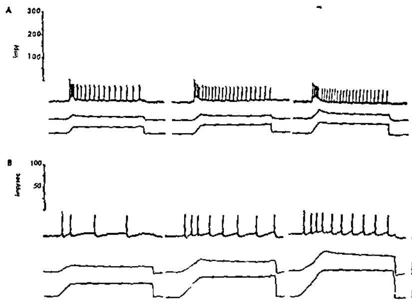


Fig 4 Impulse response and frequency of spindle *in situ* (A) and of isolated spindle (B) during stretches of increasing amplitudes Tension development shown by middle trace Upper vertical bar in *A* 0 1 mV in *B* 0 2 mV Middle vertical bar in *A* 50 mg in *B* 5 mg Lower vertical bar in *A* 2 mm in *B* 50 μm Length of preparation in *A* 19 mm in *B* 650 μm Time bar 100 ms

The velocity responsiveness of the two preparations is approximately the same as illustrated by the records in Fig 3 For different velocities of stretch the responses increase in both preparations in direct relation to the velocity of the applied stretch Following completion of the dynamic phase of the stretch the responses fall to approximately the same frequency levels

It was one of the aims of the present study to see to what extent the force transmitting structures distorted the stimulus to the sensory endings of the spindle *in situ* One way of obtaining information about this is to compare the tension of the whole muscle and the response of the spindle *in situ* during stretch It may be assumed that under otherwise similar conditions stretch stimuli applied to the isolated spindle are little distorted as compared with a stimulus to a spindle *in situ* applied as a stretch of the whole muscle Any bias in the response of the spindle *in situ* in comparison with the response of the isolated spindle would be caused by the force transmitting intrafusal and extrafusal fibres The mechanical actions of the intrafusal fibres would remain unknown in the *in situ* preparation since their tension is masked by the extrafusal tension One way in which some indirect evidence of the intrafusal tension still could be obtained would be by comparing the response of the spindle *in situ* with that of the isolated spindle The tension response relationship of the spindle has already been reported (Husmark and Ottoson 1970)

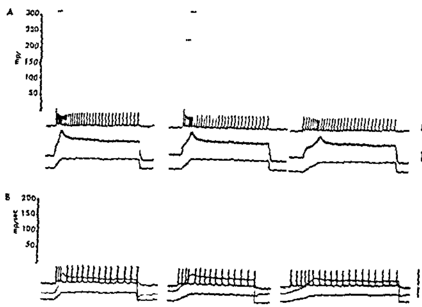


Fig 5 Impulse response and frequency of spindle in situ (A) and of isolated spindle (B) during stretches of decreasing velocities *Tension development shown by middle trace* Upper vertical bar in *A* 0 2 mV in *B* 2 mV Lower vertical bar in *A* 50 *mg* in *B* 5 mg Length increase in *A* 13 % in *B* 25 % Time bar 100 ms

In general the tension development of the whole muscle (Fig 4 A) was similar to the tension change of the isolated spindle (Fig 4 B) during stretch The frequency level in the isolated preparation (B) is lower than in the *in situ* preparation (A) but this excepted the tension and the response are related in the same manner In Fig 5 the two preparations are stretched to the same levels at three different velocities Both preparations fire at high frequencies during fast dynamic stretches and at low frequencies during slow stretch and the tension changes of the two preparations are related in the same manner as the frequency to the stretch parameters The peak tensions are high during the fast stretches whereas the static tensions remain the same

During stretch of the isolated spindle the tension usually increases in a monotonic fashion to the dynamic peak The tension of the whole muscle did not increase in such a manner during dynamic stretch In Fig 5 A it can be seen that the tension initially rose very rapidly after which it increased more slowly and more like the tension of the isolated spindle (B) In the muscle in Fig 5 A the second phase of the tension increase occurred at a lengthening of about 3 % above the initial length In 14 muscles this value was found to vary between 0 2 and 3 5 % It is possible at least for the low percentages, that the hump on the tension curve corresponds to the elastic limit described by Hill (1968)

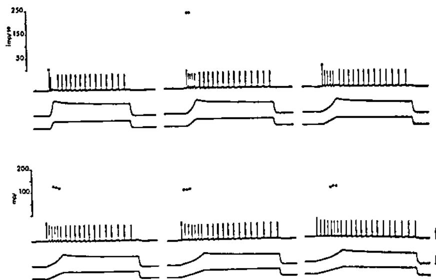


Fig 6 Impul e response and frequency of spindle *in situ* during stretches of decreasing ve locities Middle trace shows the tension development Upper vertical bar 0 5 mV [illegible] vertical bar 200 mg Length increase 10 Time bar 100 ms

The first spike of the *in situ* spindle generally coincided with or appeared just after the initiation of the second phase of the tension curve The following dynamic spikes usually showed a regular increase in frequency during the ensuing part of the dynamic stretch and both the peak frequency and the peak tension decreased [illegible] decreasing velocity (*cf* Fig 5 A) In this respect the responses of the two [illegible] tions were closely alike (*cf* Fig 5 B)

In some preparations of the spindle *in situ* the dynamic frequency and the [illegible] tension did not follow each other It is seen in Fig 6 that the dynamic [illegible] decreases although the dynamic stretch continues and the muscle [illegible] This response pattern was generally not seen in the isolated pre[illegible] the static frequencies are the same in the *in situ* spindle it can be [illegible] undamaged It should also be noted in Fig 6 that the muscle tensi[illegible] tion was rather high This high tension however did not seem [illegible] prerequisite for the decrease in dynamic frequency since in [illegible] spindles produced the same frequency pattern at low extrafusal [illegible]

It is a common feature of both the whole muscle and th[illegible] spindle that there is first a rapid and then a slow fall of the tension during [illegible] stretch In an earlier paper (Husmark and Ottoson 1971) attention was focused [illegible] qualitative similarities between the fall of the tension and the [illegible] the receptor potential The results of the study suggested that the two [illegible] not closely connected to each other since during a stretch which gave a [illegible]

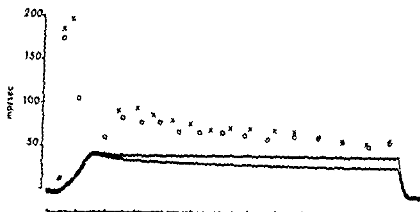


Fig 7 Instantaneous frequencies of spindle *in situ* and tension of muscle during stretch to constant length and to constant tension Circles frequency during constant stretch Crosses frequency during constant tension Time marks 20 ms

change most of the adaptation still remained In the present study the whole muscle was stretched in such a manner as to abolish the fall of the tension during maintained stretch Fig 7 shows two superimposed curves from one of these experiments It can be seen that there is very little difference in frequency during adaptation in the two circumstances About 160 ms after the dynamic stretch the frequencies are the same for the spindle although the muscle tension differs considerably It would seem that the spindle adapts at about the same rate during step stretch and step tension changes

Discussion

In *m ext long dig IV* of the frog 2—3 thin *intrafusal muscle bundles run all along the muscle* Each *intrafusal bundle consists of 3—12 fibres which have a length of* about 15—20 mm *(Gray 1957)* The fibres are of 2 types 'twitch and intermediate of which the latter type has a diameter range of 4—8 μm as compared with a range of 3—10 μm for all intrafusal fibres (Page 1966) Along the intrafusal bundle there are 1—3 encapsulated sensory regions where the afferent nerves terminate (Barker and Cope 1969) An isolated spindle is a dissected single encapsulated region with 100—300 μm of supporting intrafusal fibres and with the afferent nerve (Katz 1950 a Ottoson 1961) In this preparation the intrafusal fibres function only as a passive frame work for the sensory endings Stretch stimuli to the isolated spindle are therefore relatively directly transferred to the nerve endings For the spindle *in situ* the stimulus in terms of passive stretch of the muscle might be distorted during the transmission through the extra and intrafusal fibres (PBC Matthews 1963) Kirkwood (1972) has shown that the spindle *in situ* responds in essentially the same manner as the cut down spindle during relatively slow stretches but little is known about the response of the spindle *in situ* as compared with the response of the isolated spindle during fast stretch

The results of the present study provide additional evidence that in general the response of the spindle is regularly related to the length of both the whole muscle and the isolated encapsulated region. Some differences were noticed. One dissimilarity concerned the response frequencies during stretches of comparable strengths and velocities. When the muscle was lengthened by approximately 7—15 % a peak frequency of 175—300 imp/s was obtained whereas a relative lengthening of the isolated spindle by up to 25—30 % above the initial length resulted in a peak frequency of only 125—175 imp/s. This finding may not seem to agree with the results of Smith (1972) from experiments on the muscle spindle of the *Xenopus laevis*. He found a low sensitivity in the semi-isolated spindle to passive stretch of the muscle. It is possible that the great sensitivity of the present *in situ* preparation was due to the relatively high stretching velocity used. Stretches of low amplitudes gradually rising over 2—4 s would probably cause very little dynamic response.

A possible reason for the greater sensitivity of the spindle *in situ* could be that the muscle and the spindle were pre-stretched to a higher resting tension than the isolated spindle. This assumption is partly contradicted by the fact that the muscle length in the present study often was kept slightly below the *in situ* length. When the muscle had been dissected free it shortened spontaneously from the resting length to the equilibrium length. It is likely that not only the extrafusal but also the intrafusal fibres shortened since attempts to stretch the muscle in one step back to its *in situ* length made the spindle fire at high resting frequencies. Therefore the muscle was stretched in small steps until the spindle had attained a steady resting frequency of 1—3 imp/s which was about the same as in the isolated spindle. Under these conditions the length of the muscle was usually slightly less than the length *in situ*.

The greater sensitivity of the spindle *in situ* could also be caused by a more efficient stretch of the sensory region by non-yielding living intrafusal fibres. It is possible that cutting the intrafusal fibres of the isolated spindle changes their mechanical properties so that the same amount of stretch causes less deformation and gives a lower impulse frequency in the isolated preparation than in the spindle *in situ*.

Another possible reason for the greater sensitivity of the spindle *in situ* might be that it is subjected to influence arising from length changes in the extrafusal tissues and related to their mechanical properties. Such mechanisms have been studied in the mammalian spindle by Bridgman and Eldred (1964). They suggested that pressure on the spindle from surrounding contracting extrafusal fibres were of importance for a correct interpretation by the spindle of the mechanical state of the muscle.

In order to assess the mechanical qualities of the extrafusal fibres the tension of the whole muscle was recorded. Measurements of the tension would include the changes of the intrafusal fibre tension but since the intrafusal have a very small cross section area as compared with the extrafusal tissues the intrafusal tension is masked by the whole muscle tension change. It seems from the present study that the response is similarly related to the tensions in both preparations. This fact would

indicate that the extrafusal and the intrafusal tensions change roughly in parallel during passive stretch

In some spindles the dynamic extrafusal tension and the frequency of the spindle *in situ* changed in opposite directions *i e* the frequency was decreasing while the tension was increasing This kind of spindle response has been found previously in the primary ending of the mammalian spindle B H C Matthews (1933) ascribes the dynamic fall partly to an adaptation of the end organ P B C Matthews (1963) suggests that this non linear behaviour may be due to the experimental conditions and does not occur physiologically Hasan and Houk (1972) propose that the non linear behaviour is due to a rupture of bonds in the intrafusal fibres which would correspond to the elastic limit of Hill (1968) It would seem that the divergency of the frequency and tension curves could be explained if there were an initial mechanical overloading of the sensory endings in the elastic reticular zone during rapid stretch due to the non yielding properties of the viscous polar zones of the intrafusal fibres in these particular spindles (It is conceivable that the two intrafusal fibre types (Page 1966) have dissimilar mechanical properties If this were true the preponderance of one type over the other would determine some of the response characteristics of the spindle) The sensory endings would be much deformed initially but gradually there would be a yield of the polar parts of the muscle fibres and the deformation would become less prominent although stretching continued and the extrafusal tension rose The recordings in Fig 6 demonstrate the relative mechanical independence of the intrafusal fibres with regard to the extrafusal fibres of the muscle in which physiologically the extra and intrafusal fibres share the same efferent innervation As stretching is made more slowly the resistance to stretch would not be so great initially and the tensions in the two fibre types would increase in parallel again

In an earlier work on the isolated spindle (Husmark and Ottoson 1971) the adaptation of the spindle after a step tension change was compared with the adaptation of the same spindle after a step length change It was found that only 15- 20 % of the adaptation could be related to the overshoot of the tension In the present study the whole muscle was stretched to a constant tension and the adaptation was compared with the adaptation of the spindle after a stretch to constant length It was found that an elimination of the fall of the tension of the extrafusal muscle was reflected by the adaptive behaviour of the spindle *in situ* only to a small degree

In conclusion the relation between the parameters of lengthening or tension and response of the isolated spindle and the spindle *in situ* is generally the same Furthermore the results suggest that the dynamic response characteristics and the adaptation of the spindle during passive stretch of the muscle is relatively independent of extrafusal tissue tension From this observation follows that the deformation of the sensory endings is the result mainly of stretching of the intrafusal fibres

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References

ADRIAN E D and Y ZOTTERMAN The impulses produced by sensory nerve endings Part 2 The response of a single end-organ. *J Physiol* (Lond) 1926 *61* 151—171

BARKER D and M COPE Tandem muscle spindles in the frog *J Anat* 1962 *96* 49—56

BRIDGMAN C F and E ELDRED Hypothesis for a pressure sensitive mechanism in muscle spindles *Science* 1964 *143* 481—482

BRONK, D W Fatigue of the sense organs in muscle *J Physiol* (Lond) 1929 *67* 270—281

BROWN M C The responses of frog muscle spindles and fast and slow muscle fibres to a variety of mechanical inputs *J Physiol* (Lond.) 1971 *218* 1—17

GRAY E G The spindle and extrafusal innervation of a frog muscle *Proc Roy Soc* Ser B 1957 *146* 416—430

HASAN Z and J C HOUK Nonlinear behaviour of primary spindle receptors in response to small slow ramp stretches *Brain Research* 1972 *44* 680—683

HILL D K Tension due to interaction between the sliding filaments in resting striated muscle The effect of stimulation. *J Physiol* (Lond) 1968 *197* 637—684

HUSMARK I and D OTTOSON Relation between tension and sensory response of the isolated frog muscle spindle during stretch *Acta physiol scand* 1970 79 321—334

HUSMARK I and D OTTOSON The contribution of mechanical factors to the early adaptation of the spindle response *J Physiol* (Lond) 1971 *212* 577—592

ITO F Generator potentials of stretch receptors in the frog Sartorius muscle *Proc Japan Acad* 1968 *44* 852—855

JAHN S A A Static elasticity of isolated muscle spindles of the frog and tension development of their intrafusal muscle fibres *Acta physiol scand* 1968 *74* 384—393

KATZ B Action potentials from a sensory nerve ending *J Physiol* (Lond) 1950 a *111* 248—260

KATZ B Depolarization of sensory terminals and the initiation of impulses in the muscle spindle *J Physiol* (Lond) 1950 b *111* 261—282

KIRKWOOD P A The frequency response of frog muscle spindles under various conditions *J Physiol* (Lond) 1972 *222* 135—160

MATTHEWS B H C The response of a single end organ *J Physiol* (Lond) 1931 *71* 64—110

MATTHEWS B H C Nerve endings in mammalian muscle *J Physiol* (Lond) 1933 *78* 1—53

MATTHEWS P B C The response of de efferented muscle spindle receptors to stretching at different velocities *J Physiol* (Lond) 1963 *168* 660—678

OTTOSON D The effect of acetylcholine and related substances on the isolated muscle spindle *Acta physiol scand* 1961 53 276—287

OTTOSON D and C M SHEPHERD Transducer properties and integrative mechanisms in the frog's muscle spindle *Handbook of Sensory Physiology* Ed W R Loewenstein 1971 *I* 442—499

PAGE S G Intrafusal muscle fibres in the frog *J Microscopie* 1966 5 101—104

SMITH R S Sensory transduction in frog muscle spindles role of the intrafusal muscle fibres In Research in muscle development and the muscle spindle Ed B Q Banker et al *Excerpta Medica* 1972 353—362

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Vagal Relaxation of the Stomach Induced from the Gastric Antrum

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Abstract

ABRAHAMSSON H *Vagal relaxation of the stomach induced from the gastric antrum* Acta physiol scand 1973 *89* 406—414

The effect of mechanical stimulation of the gastric antrum on gastri (corpus-fundus) motility was studied in anesthetized cats Antral distension elicited a pronounced corpus-fundus relaxation which was not blocked by atropine guanethidine or by cervical spinal cord transection but was abolished by blockade of the vagal nerves The "threshold stimulus for eliciting the reflex response was an antral distension pressure between 5 and 10 cm H_2O Stepwise increases of antral distension produced correspondingly increased corpus fundus relaxations A steady antral distension could keep the corpus fundus part relaxed for at least 10 min In a infusion of acetylcholine to the antrum produced antral contractions followed by a marked corpus-fundus relaxation which persisted after guanethidine Electric afferent stimulation of antral nerve branches elicited a gastric relaxation similar to the corpus fundus response to antral distension —It is concluded that activation of slowly adapting tension receptors located in the antral gastric wall and with vagal afferents elicits gastric relaxation by a reflex activation of the vagal non adrenergic relaxatory fibres to the stomach These reflex mechanisms are suggested to be involved in the control of gastric reservoir function (receptive relaxation) and gastric emptying Some effects of vagotomy on gastric motility are discussed in relation to the present findings

Extrinsic reflexes emanating from receptor mechanisms in the alimentary tract are generally held to be important in regulation of gastric motility In this respect the engagement of various intestinal (*cf* Thomas and Baldwin 1968) and esophageal (Abrahamsson and Jansson 1969) receptors has been experimentally established The well documented gastric receptors on the other hand have not yet been shown to be engaged in the reflex control af gastric motility in non ruminants though a vago-vagal gastro gastric relaxatory reflex was recently demonstrated with the vagal non adrenergic relaxatory fibres as the efferent link (Abrahamsson and Jansson 1973) Evidence was presented to indicate that such relaxatory reflexes could be activated by distension of the gastric wall but the exact events could not be analysed since gastric distension and recording of the reflex response were made with the same balloon It was therefore considered of interest to gain further information about gastro-gastric reflex mechanisms concerning *e g* the properties and the site of the receptors and whether this site was separated from the site of the reflex motility response

Experience from preliminary acute experiments in cats suggested that any rough handling of the gastro-intestinal wall in connection with the preparation must be avoided in studies of vagal gastro-gastric reflex mechanisms because such procedures regularly result in a grossly reduced gastric tone. Further, the fact that the gastric antrum has a rich sensory supply (Paintal 1954, Iggo 1955, 1957 a) was thought to be of particular interest for more detailed studies of gastro-gastric reflex mechanisms. For such reasons the present investigation was so designed as to minimize abdominal surgery and, further, to explore whether selective activation of antral mechanoreceptors induced any vago-vagal reflex influences on corpus-fundus motility. A preliminary report concerning part of the present results has been presented earlier (Abrahamsson 1973 a).

Material and methods

18 cats weighing 2.0—4.8 kg and deprived of food for 24—36 h were used for the experiments. After induction with ether they were anesthetized by i.v. injection of chloralose (50—60 mg/kg b.w.). A tracheal cannula was inserted to secure free air passage.

In 4 expts gastric motility was recorded as described by Jansson (1969) with one large balloon placed in the stomach *via* the esophagus and connected to a wide waterfilled reservoir coupled to a float recorder. Thereby changes in volume of the stomach could be recorded at a low pressure set at 4—8 cm H_2O.

In 14 expts, where the effect of mechanical stimulation of the gastric antrum was studied, an abdominal wall incision was made and one small and one large balloon were introduced *via* the esophagus. By careful manual guiding through the incision the smaller condome balloon was placed in the gastric antrum and the larger rubber balloon in the corpus-fundus part. To secure their positions a ligature was cautiously placed around the pylorus just so tightly as to prevent the antral balloon to slide into the duodenum while another similar ligature hindered the antral balloon to be displaced in orad direction. The latter ligature was placed at the incisura, great care being taken to minimize interference with the blood vessels and nerves radiating transversely over the stomach. The corpus-fundus balloon was used for recording of corpus-fundus motility as described above, i.e. volume recording at a low constant pressure load.

The antral balloon, which had a capacity of 20—25 ml, was connected by an esophageal catheter to an adjustable waterfilled reservoir whereby graded antral distensions could be performed at pressures between 5—50 cm H_2O. This reservoir was also connected to a float recorder so that the volume of the antral balloon could be measured.

In a few experiments close intra-arterial infusions of acetylcholine to the antrum were made by means of a thin polyethylene catheter introduced into the right gastroepiploic artery as described by Daniel (1966). Acetylcholine was infused for about 60 s in a dose of 0.25—1.0 μg. In these experiments the catheter to the antral balloon was connected to a mercury manometer requiring a volume change of only 1 ml for a pressure change of 50 mm Hg.

In 4 experiments electrical stimulations of the central end of cut nerve branches emanating either from the gastric corpus region or the antral part were made by bipolar silver electrodes. Stimulation parameters were 1—30 Hz, 0.01—3 ms and 5—8 V. For the analysis of the reflex pathways involved vagal activity could be blocked at the cervical level either by nerve section or by local cooling with metal tubes perfused with a mixture of ice-water and alcohol. When transections of the spinal cord were performed these were placed at the C6 level.

The reflex corpus-fundus relaxation as induced from the gastric antrum was compared with the effects induced by activation of the efferent vagal relaxatory fibers to the stomach. These vagal fibres were activated either reflexly by transient distensions of a rubber balloon (14—20 ml) placed in the thoracic esophagus (*cf.* Abrahamsson and Jansson 1973) or directly by electric stimulation of the cut vagal nerve. Stimulations were then made by bipolar silver electrodes at 0.5—8 Hz, 2—3 ms and 5—8 V.

Atropine (Atropine sulphate, Merck) was administered in a dose of 0.5—1.0 mg/kg b.w. and guanethidine (Ismelin ®, CIBA) in a dose of 3.0—4.2 mg/kg b.w. In a few experiments gallamine triethiodide (Flaxedil ®, May & Baker) was used to prevent disturbing somatic motor movements. In these particular animals as well as in the spinal preparations artificial respiration was maintained by a respiration pump. Blood pressure was throughout monitored by a mercury manometer connected to the femoral artery.

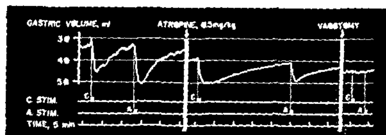


Fig 1 Cat 2 0 kg Gastric relaxation elicited by el ctric stimulations of the central ends of two small nerve branches one emanating from the gastric antrum (A STIM) and the other from the corpus part of the stomach (C STIM) The responses persist after administration of atropine 0 5 mg/kg (middle panel) but are abolished by vagal section at the cervical level (right panel) Nerve stimulation at 10 Hz 1 ms and 5 V

Results

I Afferent electric stimulation of antral nerve branches

As shown in Fig 1 (left panel) afferent antral nerve stimulation promptly elicits a long lasting gastric relaxation with similar appearance as that produced by afferent corpus nerve stimulation (*cf* Abrahamsson and Jansson 1973) The relaxatory gastric response to afferent stimulation of antral nerves persisted after administration of atropine (Fig 1, middle panel) and also after transection of the spinal cord at ... level The relaxatory gastric responses were however completely abolished by bilateral cervical vagotomy (Fig 1 right panel) The results thus show that these stimulations of afferent nerves from the antral and gastric corpus regions induce vago vagal gastric relaxation by a reflex activation of the vagal relaxatory fibres to the stomach

Afferent electric stimulation of antral nerves with varying frequencies at ? ms and 6 V for 1 min revealed that a clearcut long lasting gastric relaxation could be elicited at frequencies as low as 1 Hz With increasing frequencies the response increased up to about 10 Hz when maximal gastric relaxation was reached Afferent stimulation of antral nerves with varying impulse duration at 5—6 V and 10 Hz showed that the threshold intensity for elicitation of the described reflex relaxatory response was reached at a pulse duration of 0 1—0 2 ms

II Distension of the gastric antrum

Corpus fundus relaxation by antral distension and nervous mechanisms involved To explore whether the gastric relaxatory reflex of antral origin described above could be elicited from antral mechanoreceptors the effects of graded antral distension on corpus fundus motility were studied Within 5—8 s antral distension elicited a pronounced and long lasting corpus fundus relaxation (Fig 2 panel A) with similar appearance as that produced by direct antral nerve stimulation Furthermore this reflex response to antral distension persisted after administration of atropine (Fig 2

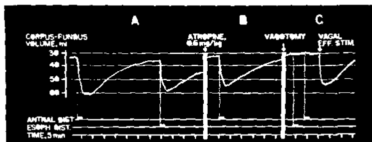


Fig 2 Cat 3 l kg Reflex relaxation of the corpus fundus part of the stomach induced by distension of the gastric antrum by a pressure of 25 cm H O A Antral distension elicits a corpus-fundus relaxation with the same appearance as that induced by distension of the esophagus with 16 ml B After administration of atropine 0 6 mg/kg antral distension still produces corpus fundus relaxation C Vagal section in the neck abolishes the corpus fundus response to antral as well as esophageal distension Electric stimulation of the distal vagal ends (2 Hz 2 ms 8 V) induces a corpus fundus relaxation similar to that obtained by antral distension

panel B) and guanethidine as well as after cervical spinal cord transection The response was however completely or in some experiments almost completely (see below) abolished by cervical vagal blockade (Fig 2 panel C) It was also observed that the reflex corpus fundus response to antral distension closely mimicked that induced by activation of the vagal relaxatory fibres whether reflexly by esophageal distension (Fig 2 panel A) or by direct efferent vagal stimulation (Fig 2 panel C) The results thus show that antral distension elicits corpus fundus relaxation by a reflex activation of the vagal relaxatory nerve fibres This is further supported by the observation that a sustained reflex corpus fundus relaxation induced by continuous antral distension was interrupted if vagotomy was performed (Fig 3)

In some of the non atropinized cats rhythmic corpus fundus contractions were present even during vagal blockade When this was the case antral distension promptly inhibited the rhythmic contractions despite the absence of any extrinsic vagal influence on the stomach This inhibitory mechanism is further analysed in a subsequent report (Abrahamsson 1973 b)

Effects of variation in degree and duration of the antral distension To study the stimulus response characteristics of the vagally mediated reflex corpus fundus relaxation stepwise increases of antral distension were made in 6 atropinized cats Occasionally a reflex response was obtained already at an antral distension pressure of 5 cm H O and an antral pressure of 10 cm H O always elicited a clearcut corpus fundus relaxation (Fig 3 and 4) Stepwise increases of antral distension led to corresponding increases in corpus fundus volume as shown in Fig 3 and 4

When the antrum was kept constantly distended for as long as 10 min in atropinized cats there was never any tendency for the reflex corpus fundus relaxatory response to escape As shown in Fig 3 a steady antral distension by a pressure e g of 20 cm H O produces a continuous relaxed or even accentuated relaxing state in the corpus fundus A regain of corpus fundus tone occurs first when vagotomy is

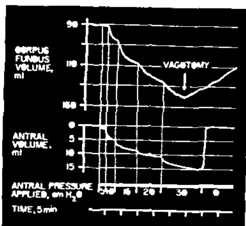


Fig 3

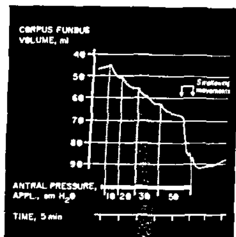


Fig 4

Fig 3 Cat 3 7 kg Atropine 0 5 mg/kg Gradually increased corpus fundus relaxations induced reflexly by stepwise increases of antral distension induced by pressures from 5 to 30 cm H_2O The corpus fundus relaxation appears when the antral pressure is increased from 5 to 10 cm H_2O Vagal section during the antral distension is immediately followed by a regain of corpus fundus tone which is not affected by deflation of the antrum. Corpus fundus volume is recorded at a pressure of 6 cm H_2O

Fig 4 Cat 3 1 kg Atropine 0 5 mg/kg Graded corpus fundus responses to stepwise increases of antral pressure from 10 to 50 cm H_2O A series of spontaneous swallowing movements appears during the highest level of distension and is accompanied by a further marked corpus fundus relaxation in addition to that already obtained.

performed Thus the antral receptor mechanism involved in this vagal relaxatory reflex seems to be a slowly adaptive one

Quantitative aspects of the reflex corpus fundus response The corpus fundus relaxatory response to antral distension was ordinarily pronounced already at moderate degrees of distension the corpus fundus volume being often doubled by an antral distension at 25—30 cm H_2O The relative magnitude of the volume increase was higher the higher the prevailing corpus fundus tone However even pronounced antral distensions never produced *maximal* corpus fundus relaxations as is illustrated in Fig 4 Here the corpus fundus is reflexly relaxed by an antral distension pressure of 50 cm H_2O In this situation the animal exhibited a series of spontaneous *swallowing movements known to induce reflex activation of the vagal relaxatory fibres* (Abrahamsson and Jansson 1969) which immediately led to a marked accentuation of the reflex corpus fundus relaxation

III *Close i a infusion of acetylcholine to the antrum*

Acetylcholine (Ach) was in some experiments i a infused to the antrum and produced antral contractions followed by a long lasting corpus fundus relaxation closely mimicking that produced by e g reflex activation of the vagal relaxatory fibres by esophageal distension (Fig 5) Even when the antral balloon is empty Ach injection (1μg in 1 min) to the antrum induces a clearcut but *moderate* corpus-

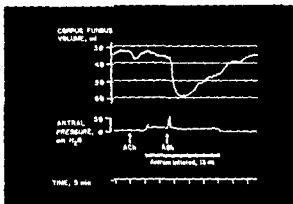


Fig 5 Cat 4 8 kg The antral balloon connected to a mercury manometer Corpus fundus relaxation induced by i a infusion of acetylcholine to the antrum (Ach 1 μg in 1 min) When the antrum is deflated the response to the drug is of small magnitude When infused to the inflated antrum Ach produces a marked antral pressure increase and a very pronounced corpus fundus relaxation The absence of any change in antral pressure recording at the first Ach infusion is probably due to the recording method Note the close similarity between the corpus-fundus response obtained here and that illustrated e g in Fig 2 Note further the acceleration of the corpus fundus recovery phase upon deflation of the antrum

fundus relaxation On the other hand the same Ach injection during a prevailing slight antral distension produces powerful antral contractions that are promptly followed by a profund corpus fundus relaxation It is also observed in Fig 5 that the antrum inflation of 13 ml *per se* produces a slight corpus fundus relaxation and that subsequent antral deflation is followed by an acceleration of the slow recovery phase after the previous corpus-fundus relaxatory response These corpus fundus relaxations upon Ach administration to the antrum were unaffected by guanethidine while atropine abolished the antral response to Ach and consequently the corpus fundus response as well

Discussion

The present investigation has provided evidence that the antral part of the stomach contains mechanoreceptors which can produce vago vagal reflex relaxation of more proximal gastric parts *via* the vagal non adrenergic relaxatory fibres This was clear from the fact *first* that the reflex response was not blocked by atropine or guanethidine cf Martinson 1965 Campbell 1966) but by vagal blockade and *second* that it was closely mimicked by either the response to esophageal distension known to reflexly activate the relaxatory vagal supply to the stomach (Abrahamsson and Jansson 1969 or that produced by efferent vagal stimulation

Except for studies in ruminant animals (see Leek 1971) few data are available concerning reflex gastric motility responses to mechanical gastric stimulation Grigg and Evans (1960) reported that stretching of the pylorus with a mechanical clip produced gastric relaxation in the cat The authors concluded that the vagus constituted the afferent reflex link but not the efferent link The mode of reflex elicitation and the way in which the reflex arc was analysed in the present study make comparisons difficult with the present study

The present results where gastric vagal branches were stimulated

suggest that receptor mechanisms capable to produce vago-vagal gastric relaxation of the abovementioned type exist both in proximal and distal parts of the stomach (*cf* Abrahamsson and Jansson 1973) It was also found that the electrical stimulation threshold for the gastric afferents involved was of the same order as that of the non medullated vagal heart afferents involved in gastric relaxatory reflexes (Abrahamsson and Thoren 1972) These observations are in accordance with the findings that 98—99 % of the afferent fibres in the abdominal vagus of the cat are non medullated (Agostoni *et al* 1957)

To understand the possible role of the presently described antral reflexes for the regulation of gastric motility one must consider the type of antral stimuli capable to elicit the reflex corpus-fundus relaxation. In several studies 2 different types of gastric mechanoreceptors have been recognized in electrophysiological studies of vagal afferents

1 Slowly adapting mechanoreceptors which show a steady high frequency discharge upon sustained gastric distension (Paintal 1954 Iggo 1955 1957a Leek 1969 Leek 1971 Harding and Leek 1972) These receptors are activated both by passive distension and by contraction of the stomach and are therefore considered to be *in series* with the smooth muscle cells in the gastric wall thus functioning as "tension receptors

2 Rapidly adapting mechanoreceptors located in or near the gastric mucosa and activated by both mechanical and certain chemical stimuli (Iggo 1957b Harding Leek 1972 Davison 1972 This latter receptor type responding to stroking of the mucosa can give *on* and *off* response when a steady mechanical stimulus is applied but no response while the mechanical stimulus is held steady

For such reasons it seems reasonable to assume that it is the slowly adapting type of tension receptors that are responsible for the longlasting steady relaxation obtained in the corpus-fundus region during prolonged antral distension

Close i.a injection of acetylcholine (Ach to the antrum caused forceful antral contractions rapidly followed by pronounced relaxations of the corpus-fundus region. This corpus-fundus relaxation can be explained as a reflex response due to activation of antral tension receptors as a result of antral smooth muscle contractions. Such a mode of reflex elicitation is also suggested by the observation that Ach produced larger reflex responses when injected to the inflated antrum because according to the law of Laplace the antral contractions then led to a higher wall tension. At present it may be difficult to prove beyond doubt that also normal antral gastric contractions can reflexly activate the vagal relaxatory fibres because atropine must be given to demonstrate their involvement and atropine will of course also interfere with the gastric contractions. One must further be aware of the possibility that Ach may excite sensory endings directly or sensitize them to the natural stimulus (Paintal 1964 However the effects of Ach injection to the antrum strongly indicate that antral receptors exist which elicit a non adrenergic relaxation of the corpus-fundus region with an appearance that in detail mimicks that induced by activation of the vagal relaxatory fibres to the stomach

The mode of initiation of the vagal relaxatory reflexes described above suggests their involvement in important aspects of gastric motility control Thus activation of the antral mechanoreceptors by the gastric contents may considerably contribute to the control of the reservoir capacity in receiving further volumes without substantial rises in intragastric pressure In this respect the reflexes from the antrum may work together with vago-vagal relaxatory reflexes elicited from pharynx and esophagus (Abrahamsson and Jansson 1969) and also from more proximal parts of the stomach (Abrahamsson and Jansson 1973)

Further vago-vagal antro-gastric relaxatory reflexes may well be activated whenever the antrum is involved in the transport of gastric contents Such an involvement of this reflex mechanism will decrease the force whereby contents are transported from the corpus fundus part to the antral pump (Cannon 1898) decreasing the caudad gastric pressure gradient (*cf* Nelsen and Kohatsu 1971) and consequently delaying gastric emptying Reported effects of vagotomy in man are in accordance with these suggestions insofar as vagotomy leads to an increased gastric tone (Rowe *et al* 1952 Aune 1969 Koster and Madsen 1970) as well as to an increased gastric emptying rate as a result of so called selective proximal vagotomy (Moberg *et al* 1972)

The low degree of antral distension capable to elicit reflex corpus fundus relaxation seems to be far below what is considered as pathophysiological distensions In studies on antral distension in dogs Goldberg (1931) found that vomiting was produced first at distending pressures above 40 cm H_2O The present results show that an antral distending pressure of only 5—10 cm H_2O is enough to elicit reflex corpus fundus relaxation Upon intense antral distension this reflex response was sometimes accentuated in association with swallowing (Fig 4) and it could then either be an effect of normal swallowing movements *per se* or due to an induced reflex vomiting response where the somatomotor component was depressed by anesthesia In both these situations the vagal relaxatory fibres are reflexly activated (Abrahamsson and Jansson 1969 Abrahamsson and Thoren 1973) However the present results suggest that even an intense antral distension does not produce any *maximal* corpus fundus relaxation but further relaxation can be elicited if the vagal relaxatory fibres are further activated due to other types of reflex influences

Besides the gastric mechanoreceptors dealt with in the present investigation other mechanoreceptors are present in the stomach During the course of the present experiments it was found that mechanical antral stimulation affected corpus fundus motility also *via* a reflex mediated by the splanchnic adrenergic nerves This latter reflex mechanism and its functional relationship to the reflexes presented here will be discussed elsewhere (Abrahamsson 1973 b)

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References

ABRAHAMSSON H Reflex suppression of gastric motility elicited from antral mechanoreceptor *Acta physiol scand* 1973 a *87* 40 A

ABRAHAMSSON H Reflex adrenergic inhibition of gastric motility elicited fom the gastri *scand* 1973 *88* 289—295

ABRAHAMSSON H and G JANSSON Elicitation of reflex vagal relaxation of the stomach fron pharynx and esophagus in the cat *Acta physiol scand* 1969 *77* 172—178

ABRAHAMSSON H and G JANSSON Vago vagal gastro gastric relaxation in the cat *Acta physiol* antrum *Acta physiol scand* 1973 b *89* In press

ABRAHAMSSON H and P THORÉN Reflex relaxation of the stomach elicited from receptor located in the heart An analysis of the receptors and afferents involved *Acta physiol scand* 1972 *84* 197—207

ABRAHAMSSON H and P THORÉN Vomiting and reflex vagal relaxation of the stomach elicited from heart receptors in the cat *Acta physiol scand* 1973 *88* 433—439

AGOSTONI E J E CHINNOCK M DE BURGH DALY and J G MURRAY Functional and hi tological studies on the vagus and its branches to the heart lungs and abdominal viscera in the cat *J Physiol* (Lond) 1957 *135* 182—205

AUNE S Intragastric pressure after vagotomy in man *Scand J Gastroent* 1969 *4* 447—452

CAMPBELL G The inhibitory nerve fibres in the vagal supply to the guinea pig stomach *J Physiol* (Lond) 1966 *185* 600—612

CANNON W B The movements of the stomach studied by means of the rontgen rays *Amer J Physiol* 1898 *1* 359—382

CRAGG B G and D H L EVANS Some reflexes mediated by the afferent fibers of the abdominal vagus in the rabbit and cat *Exp Neurol* 1960 *2* 1—12

DANIEL E E Electrical and contractile responses of the pyloric region to adrenergic and cholinergic drugs *Canad J Physiol Pharmacol* 1966 *44* 951—979

DAVISON J S Response of single vagal afferent fibres to mechanical and chemical stimulation of the gastric and duodenal mucosa in cats *Quart J exp Physiol* 1972 *57* 405—416

GOLDBERG S L The afferent paths of nerves involved in the vomiting reflex induced by distension of an isolated pyloric pouch *Amer J Physiol* 1931 *99* 156—159

HARDING R and B F LEEK Gastro-duodenal receptor responses to chemical and mechanical stimuli investigated by a single fibre technique *J Physiol* (Lond) 1972 *222* 139 P—140 P

IGGO A Tension receptors in the stomach and the urinary bladder *J Physiol* (Lond) 1955 *128* 593—607

IGGO A Gastro-intestinal tension receptors with unmyelinated afferent fibres in the vagus of the cat *Quart J exp Physiol* 1957 a *42* 130—143

IGGO A Gastric mucosal chemoreceptors with vagal afferent fibres in the cat *Quart J exp Physiol* 1957 b *42* 398—409

JANSSON G Vago-vagal reflex relaxation of the stomach in the cat *Acta physiol scand* 1969 *75* 245—252

KOSTER N and P MADSEN The intragastric pressure before and immediately after truncal vagotomy *Scand J Gastroent* 1970 *5* 381—383

LEEK B F Reticulo ruminal mechanoreceptors in sheep *J Physiol* (Lond) 1969 *202* 585—609

LEEK B F Abdominal visceral receptors *Handbook of sensory physiology* Vol III Part 1 Enteroreceptors Editor E Neil Springer Verlag Berlin 1971 116—160

MARTINSSON J Vagal relaxation of the stomach Experimental re investigation of the concept of the transmission mechanism *Acta physiol scand* 1965 *64* 453—462

MOBERG S G CARLBERGER F BARANY and G LUNDH Gastric emptying in peptic ulcer patients before and after partial gastrectomy and selective proximal vagotomy *Rend c R Gastroenterol* 1972 *4* 1 —

NELSEN T S and S KOHATSU The stomach as a pump *Rendic R Gastroenterol* 1971 *3* 65—71

PAINTAL A S A study of gastric stretch receptors Their role in the peripheral mechanism of satiation of hunger and thirst *J Physiol* (Lond) 1954 *126* 255—270

PAINTAL A S Effect of drugs on vertebrate mechanoreceptors *Pharmacol* 1964 *16* 341—380

ROWE C R K S GRIMSON B H FLOWE C K LYONS F H LONGINO H M TAYLOR and N C DURHAM Early and late effects of vagotomy on gastric secretions and motility *Surgery* 1952 *32* 226—250

THOMAS J E and M V BALDWIN Pathways and mechanisms of regulation of gastric motility In *Handbook of Physiology* Sect 6 Vol IV Washington 1968 1937—1968

Acta physiol scand 1973 89 415—422

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Effects of Mg^{2+} and Ca^{2+} on Noradrenaline Release and Uptake in Adrenergic Nerve Granules in Different Media

By

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Abstract

EULER U S v and F LISHAJKO *Effects of Mg^{2+} and Ca^{2+} on noradrenaline release and uptake in adrenergic nerve granules in different media* Acta physiol scand 1973 *89* 415—422

Uptake and release of noradrenaline (NA) from a suspension of storage granules from bovine splenic nerves was measured after incubation in various media and effects of added Mg^{2+} and Ca^{2+} observed No significant differences in NA release rate were observed in different media when NA uptake was prevented by addition of potassium ferricyanide to the medium Uptake was facilitated by phosphate ions 5—30 mM but counteracted by monovalent cations In sucrose phosphate media uptake of NA almost balanced spontaneous release Mg^{2+} and Ca^{2+} had only a small action on NA release in the absence of NA uptake In sucrose phosphate media Mg^{2+} facilitated uptake whereas Ca^{2+} enhanced NA release This action was associated with the formation of a calcium phosphate precipitate No release or uptake of NA was observed during incubation at ice water temperature nor did Mg^{2+} or Ca^{2+} have any effect The results tend to show that release and uptake of NA in storage granules are separate temperature dependent processes influenced by mono- and divalent cations and phosphate ions

A suspension of adrenergic nerve granules prepared from bovine splenic nerves gives off and takes up noradrenaline (NA) at rates depending *inter alia* on incubation temperature pH and NA concentration in the medium In a NA free medium containing 130 mM potassium phosphate at pH 7 the half time for release is approximately 35 min at 20° (Euler and Lishajko 1967) With the standard technique used the NA level in the incubation medium assumes levels of 1—3 μM The ensuing uptake of NA reduces the net release rate corresponding to an increase in half time to 50—75 min

In the present report the effect of different media and of addition of Mg^{2+} and Ca^{2+} on the release and uptake of NA has been studied

Methods

Bovine splenic nerves were obtained from the slaughterhouse and brought to the laboratory on ice and prepared within 1—2 h After desheathing the nerves were cut in small pieces with scissors and homogenized with an Ultra Turrax apparatus in cold media of varying compo-

sitions approximately 1 g nerve per 10 ml incubation fluid The homogenate was centrifugated for 10 min at 9000 × g in a refrigerated centrifuge and 8 ml of the supernatant containing the nerve granules used for incubation at 20 in polythene tubes After incubation the tubes were centrifuged 30 min at 50 000 × g and NA determined fluorimetrically in the sediment after extraction with 0 5 ml 0 4 M perchloric acid in the supernatant after purification on alumina

In some experiments ^{14}C-(—) NA was added to concentrations of approximately 0 2 μM and the radioactivity measured in aliquots of the purified extracts The incorporation of labelled NA was measured by determining the spec act ratio in the sediment after resu pension and recentrifugation in ice cold media without label and in the supernatant (SA_{SED}/SA_{SU}) NA free media were provided by addition of potassium ferricyanide 5 mM (Euler and Lishajko 1967)

The following media were used

Phosphate free media
270 mM sucrose
210—240 mM sucrose + 30—60 mM Tris-maleic acid buffer
160 mM KCl + 30 mM Tris-buffer
155 mM NaCl + 80 mM Tris-buffer

Phosphate containing media
130 mM K phosphate
130 mM K phosphate + 80 mM Tris buffer
30 mM K phosphate + 120 mM NaCl
30 mM K phosphate + 210 mM sucrose
30 mM K phosphate + 123 mM choline chloride
30 mM K phosphate + 123 mM KCl
5 mM K phosphate + 210 mM sucrose + 25 mM Tris-buffer

Magnesium and calcium were added as chlorides to 3 and 6 mM concentrations pH of the media was adjusted to 7 0—7.5

In the experiments where CaCl was added to pho phate containing media a flocculent precipitate or turbidity occurred This became particularly visible on adding NaOH to the purified extract for the fluorimetric determination. Before fluorimetric readings the precipitate was removed by centrifugation The occurrence or turbidity did not alter the fluorimetric readings in the centrifuged samples

In order to check non specific actions of the ions determinations of NA were also made after 60 min incubation at ice water temperature which normally is not accompanied by NA release.

Results

1 *Effect of added Mg and Ca² on NA release in NA free media*

When uptake of NA into the granules was prevented by addition of 5 mM potassium ferricyanide to the various incubation media the mean value of the remaining NA in the sediment after incubation 60 min at 20° was 26 ± 1 0 (n = 20) per cent of the original amount No consistent differences were observed in the NA content of the sediment after incubation in NA free media of different compositions In earlier experiments (Euler and Lishajko 1967) the mean remaining NA value in 130 mM potassium phosphate medium in the presence of Fe³ was slightly higher 34 per cent of the original amount after incubation

Addition of MgCl or CaCl 3—6 mM did not elicit any significant changes in the remaining NA after incubation in NA free media irrespective of the composition although there was a tendency to slightly higher values (Fig 1) Thus the mean NA content with MgCl added was 29 ± 1 7 per cent and with CaCl 28 ± 1 6 per cent of the original amount in the sediment (Table I)

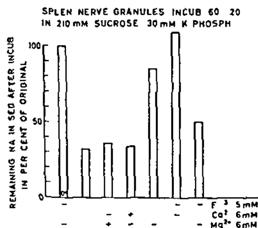


Fig 1

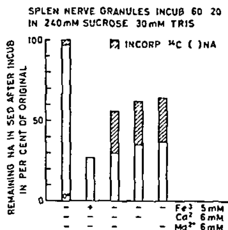


Fig.2

Fig 1 Bovine splenic nerve granules incubated 60 min at 20 in 210 mM sucrose + 30 mM K phosphate Control incubated at ice water temperature Ordinate remaining NA in sediment after incubation in percent of control The reuptake of NA in tubes 5—7 is prevented by potassium ferricyanide in tubes 2—4 $MgCl_2$ and $CaCl_2$ added as indicated

Fig 2 Bovine splenic nerve granules incubated 60 min at 20 in 240 mM sucrose + 30 mM Tris-buffer Control incubated at ice water temperature Ordinate remaining NA in sediment after incubation in per cent of control Tube 2 NA free medium. Hatched part of columns NA uptake $MgCl_2$ and CaCl added as indicated

TABLE I Per cent remaining NA in sediment after incubation 60 min at 20 C in controls and with addition of Mg and Ca (M ± S E) NA concentration in all media (except NA free) 1–3 μM n = number of expts

Incubation medium	Control	3–6 mM Mg Cl	3–6 mM CaCl
NA free medium	26 ± 1 0 (n = 20)	29 ± 1 7 (n = 6)	28 ± 1 6 (n = 6)
Isoton NaCl KCl or choline chlorid — 0 mM Tris-buffer	38 ± 2 4 (n = 5)	40 ± 2 4 (n = 5)	44 ± 2 0 (n = 5)
210–240 mM sucrose + 30 mM Tris buffer	57 ± 5 4 (n = 5)	57 ± 6 2 (n = 3)	48 ± 6 3 (n = 3)
210–240 mM sucrose + 5–30 mM phosphate	92 ± 3 5 (n = 5)	111 ± 6 8 (n = 5)	42 ± 7 2 (n = 5)
130 mM K phosphate	57 ± 1 7 (n = 37)	52 (n = 2)	48 (n = 2)
Relative uptake of NA estimated by C-NA in per cent of original amount (sucrose phosphate med)	51 ± 3 2 (n = 6)	69 ± 6 0 (n = 5)	19 ± 3 0 (n = 6)

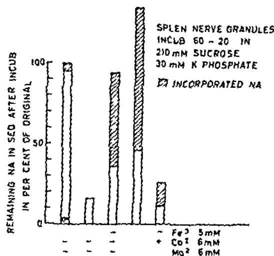


Fig 3a

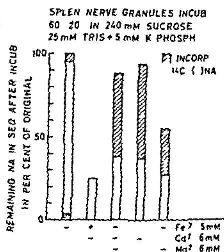


Fig 3b

Fig 3 a Bovine splenic nerve granules incubated 60 min at 20 in 210 mM sucrose + 30 mM K phosphate Control incubated at ice water temperature Ordinate remaining NA in sediment after incubation in per cent of control Tube 2 NA free medium Hatched part of columns NA uptake MgCl and $CaCl_2$ added as indicated

Fig 3 b Same as 3 a but incubation in 240 mM sucrose + 25 mM Tris mal acid buffer + 5 mM K phosphate

2 Release and uptake of NA in NA containing media

In NA containing media the amount of NA remaining in the sediment after 60 min incubation at 20° was consistently higher owing to reuptake of NA from the medium which contained 1—3 μM NA. In addition the composition of the medium greatly influenced the result (Fig 1—3)

In the phosphate free media containing isotonic NaCl or KCl the mean value of remaining NA in the sediment was 38 ± 2 4 per cent (S E n = 5) of the original amount This value is significantly lower than that obtained with isotonic phosphate alone (57 ± 1 7 Euler and Lishajko 1967) indicating a facilitation of uptake in the presence of phosphate since the basic release rate is the same

Similarly the remaining NA in the sediment after incubation with sucrose alone or together with 30 mM Tris buffer was 57 ± 5 4 per cent of the original amount (Fig 2) whereas addition of phosphate 5—30 mM increased the value to 92 ± 3 5 per cent (Fig 1 3) (Table I) From the experiments with labelled NA (see below) it was evident that the higher value depended on increased uptake of NA during incubation With 123 mM choline chloride combined with 30 mM K phosphate as medium the remaining NA in the sediment after incubation had a relative value of 54 ± 1 0, similar to that observed with 130 mM potassium phosphate alone

The results suggest a promoting effect of phosphate on NA uptake The phosphate effect can be noticed even with 5 mM concentration although it is more marked at 30 mM

Similarly a low concentration of monovalent cations such as in sucrose alone or sucrose with Tris buffer facilitates uptake which is markedly higher than in pure NaCl on KCl media In the presence of phosphate and when monovalent cations are present in low concentrations uptake is facilitated so that after 60 min incubation the remaining NA in the sediment has almost the same value as before incubation Thus the net release is less than 10 per cent depending on the efficient uptake

Effect of $MgCl_2$ and $CaCl_2$ on release and uptake of NA in different media

The effects of Mg and Ca on the net release and uptake of NA in the particles were studied in different media In KCl or NaCl without phosphate addition of Mg in concentrations of 3—6 mM caused an insignificant increase in the remaining NA level from 38 ± 2 4 per cent (control) to 40 ± 2 4 per cent. After addition of Ca 3—6 mM the corresponding value was 44 ± 2 0 per cent (Table I) In sucrose or sucrose Tris buffer but in the absence of phosphate addition of Mg had no effect whereas Ca caused a slight fall from 57 ± 5 4 per cent to 48 ± 6 3 which however with the available number of experiments did not reach significance In pure phosphate media Mg still had no overt effect whereas Ca had an effect similar to that noticed in sucrose or sucrose Tris alone

In the sucrose phosphate media Mg had a marked action which resulted in an average increase in the NA in the sediment from 92 ± 3 5 to 111 ± 6 8 ($p < 0\ 05$) per cent of the original amount (Fig 1 3) Ca on the other hand regularly caused a strong decrease in the NA content from 92 ± 3 5 to 42 ± 7 2 per cent of the original amount a result indicating either a diminished uptake or increased rate of release or both (Fig 1 3 Table I) Fairly large variations in the Ca effect were noticed as illustrated in the figures

Incorporation of labelled NA

In the experiments with labelled NA added to various media containing 5—30 mM K phosphate the mean incorporation of NA as measured by the specific activity ratio between NA in sediment and supernatant after incubation was 0 58 ± 0 023 (n = 7) of the actual amount of NA in the controls and 0 59 ± 0 033 after addition of Mg 3—6 mM Addition of Ca caused a moderate fall in the uptake to 0 50 ± 0 025

No consistent difference in these values were noted between NaCl KCl or sucrose media containing 30 mM K phosphate Sucrose Tris-media showed a somewhat lower degree of incorporation

If however the radioactive uptake is measured in per cent of the original amount of NA it is 51 ± 3 2 (n = 6) in sucrose phosphate media and 31 ± 2 6 (n = 4) in other media or about 60 per cent higher in the sucrose phosphate media as in the others containing NaCl KCl or choline chloride + 30 mM phosphate Addition of Mg further increased the uptake to 69 ± 6 0 (n = 5) per cent in sucrose phosphate media (Fig 3 Table I) Addition of Ca decreased the NA uptake to 19 ± 3 0 (n = 6) per cent a value which does not significantly differ in sucrose phosphate

and in other phosphate containing media (Fig 3) Thus the relative incorporation of NA is only moderately decreased by Ca , when referred to the remaining NA whereas it is markedly reduced in relation to the original amount of NA suggesting a rapid release of NA In sucrose Tris buffer, on the other hand Ca did not significantly alter the uptake of NA (Fig 2)

Incubation at ice water temperature

Incubation with Mg^{2} or Ca for 60 min at ice water temperature lead only to small changes in the NA content of the sediment as compared with the control irrespective of the nature of the media The same was observed on addition of ferricyanide at 0—2° in order to remove NA from the medium The results show that the effects observed with Ca (or Fe^{3}) were not due to damage of membranes or other actions independent of temperature

On addition of CaCl 3 or 6 mM to media containing phosphate turbidity or a precipitate occurred as mentioned under Methods This appeared as turbidity when the phosphate concentration was 5 mM but developed gradually as a flocculent precipitate in the media containing 30 mM phosphate No precipitate occurred on addition of MgCl Since the low NA values in the sediment after incubation with Ca always went together with an overt precipitation it is assumed that the observed effect is associated with the formation of calcium phosphate

Discussion

From the reported experiments it is evident that the rate at which NA is released from isolated adrenergic nerve granules on incubation at 20° C in a NA free medium is approximately constant in a variety of media containing either sucrose NaCl KCl choline chloride or K phosphate Addition of divalent cations like Mg or Ca has no overt action The implication is, that when uptake is prevented the NA release occurs at a rate which seems to be inherent in the particles and mainly dependent on temperature and pH of the medium *It is known however* that certain drugs apart from those damaging the granule membrane can enhance this release Of particular interest among them are some indirectly acting amines and uncouplers of oxidative phosphorylation (Euler and Lishajko 1968 1969) although most of their effect is to inhibit uptake

While the release rate for NA thus appears to be relatively unaffected by the composition of the incubation medium whether this is sucrose or potassium phosphate sodium chloride or a choline salt the uptake process is much more susceptible to influence by the medium As shown previously (Euler and Lishajko 1968) uptake of NA occurs at a rate dependent on the concentration of NA *in the medium* This uptake is greatly facilitated by ATP in the presence of Mg , which also holds for the net uptake of NA observed with adrenergic nerve granules after *previous* partial depletion (Euler and Lishajko 1963 1969)

While uptake of NA in the granules using 130 mM K phosphate as medium is subject to moderate variations in the different experiments depending on the actual NA level in the medium the present data show that it is greatly enhanced when 130 mM K phosphate is replaced by 210—240 mM sucrose and 5—30 mM phosphate This suggests that the uptake process is associated with the ionic milieu in which monovalent cations seem to reduce the uptake The higher uptake in 240 mM sucrose +Tris-buffer than in 130 mM NaCl+Tris-buffer is further enhanced by the presence of 5—30 mM K phosphate Thus the NA content in the granules may be maintained constant for 60 min at 20° during incubation in a medium consisting of 210 mM sucrose and 30 mM potassium phosphate while it is reduced to ap proximately one half in 240 mM sucrose alone or 130 mM potassium phosphate alone Even with a phosphate concentration of 5 mM the NA content is well main tained in a sucrose medium Whether the normal level of inorganic phosphate in the medium which can be estimated at approximately 1 mM influences uptake is as yet undetermined A lowered uptake is observed in washed and resuspended granules in a phosphate medium This effect may be due to absence of Mg- since a similar effect is noted in unwashed granules to which EDTA had been added

In those experiments where uptake is moderate as with sucrose and Tris buffer the unlabelled portion corresponds approximately to the part remaining after basic release (*i e* in NA free medium) This portion is usually slightly increased when uptake is greater A possible explanation for the increase is that reuptake of some of the unlabelled amine occurs immediately after release before mixing with the label in the medium

A moderate inhibitory action of Mg- on the release of catecholamines has recently been demonstrated on adrenal medullary granules (Lishajko 1970) The present observations tend to show that this may be the case also with nerve granules Thus addition of Mg- to the incubation medium in concentrations of 3 or 6 mM slightly increases the amount of NA remaining in the sediment when uptake is prevented Some of the effect of Mg is notably due to increased uptake This might be as sociated with the previously described facilitation of NA uptake by ATP and Mg-

More intricate is the finding that addition of Ca in the presence of phosphate causes an apparent increa e of NA release This is particularly striking in a sucrose medium where NA uptake normally is high when phosphate is present The relative uptake of NA measured by the ratio SA $_{Et}$/SA $_t$ is only moderately decreased by Ca in the presence of sucrose and phosphate The effect of calcium in the presence of phosphate is apparently associated with formation of calcium phosphate since signs of precipitation occur in all cases when the Ca effect has been demonstrated It is notable however that the basic release in a NA free medium is not affected by the formation of calcium pho phate When phosphate is not present Ca has no or only a weak inhibitory effect on NA uptake or release

An enhancing effect of calcium phosphate on the release of catecholamines from adrenal medullary granules has recently been described by Lishajko (1970) He noted that the effect was associated with a freshly formed precipitate of calcium

phosphate, and that it exerted the strongest action when phosphate was present in about 20 mM concentration and Ca in 3—6 mM. In this case the effect was clearly on the release since actual net uptake of catecholamines was not observed in isolated adrenal medullary particles.

It is not at present possible to make any statement about the mechanism(s) by which calcium phosphate enhances the release of NA in nerve granules during conditions when uptake normally is high. It can be assumed, however, that the freshly formed "reactive" or nascent calcium phosphate interacts with the release mechanism, presumably at the particle membrane. Interactions with proteins or lipoproteins are likely to occur at this level. Even if the mechanism remains to be elucidated it nevertheless merits special interest that Ca in the adrenergic nerve *ending as well as in the adrenal chromaffin cell is a prerequisite for the liberation* of the transmitter from its bound state. This may occur *in vivo* by release and diffusion following contact between the granule and the excited axon membrane but the present experiments appear to offer an alternative possibility *viz.* increased release rate from the storage granules possibly by interaction of temporarily mobilized calcium with phosphate at this site.

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References

EULER, U. S. v. and F. LISHAJKO, Effect of adenine nucleotides on catecholamine release and uptake in isolated adrenergic nerve granules. *Acta physiol. scand.* 1963. 59. 451—461.

EULER, U. S. v. and F. LISHAJKO, Reuptake and net uptake of noradrenaline in adrenergic nerve granules with a note on the affinity for l- and d-isomers. *Acta physiol. scand.* 1967. 71. 151—162.

EULER, U. S. v. and F. LISHAJKO, Effect of directly and indirectly acting sympathomimetic amines on adrenergic transmitter granules. *Acta physiol. scand.* 1968. 73. 78—92.

EULER, U. S. v. and F. LISHAJKO, Effect of some metabolic cofactors and inhibitors on transmitter release and uptake in isolaed adrenergic nerve granules. *Acta physiol. scand.* 1969. 77. 298—307.

LISHAJKO, F., Releasing effect of calcium and phosphate on catecholamines, ATP and protein from chromaffin cell granules. *Acta physiol. scand.* 1970. 79. 575—581.

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From the Institute of Physiology University of Helsinki Finland

Effect of Acute Exposure to Cold on Blood Thyrotrophin (TSH) and Corticosterone Concentrations in the Rabbit

By

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Abstract

Leppäluoto J H Lybeck T Ranta and P Virkkunen *Effect of acute exposure to cold on blood thyrotrophin (TSH) and corticosterone concentrations in the rabbit* Acta physiol scand 1973 89 423—428

Rabbits were exposed to various degrees of cold and plasma TSH concentration was measured by the McKenzie bioassay method (McKenzie 1958) and plasma corticosterone by a fluorometric method. After the transfer of 19 rabbits to a cold room (10 C) no consistent change ($p > 0.05$) in the plasma TSH level could be seen up to 3 h whereas the plasma corticosterone level rose transiently from 6.8 to 15.8 μg/100 ml ($p < 0.01$) at 1 h but then fell to the initial level. After immersion of anesthetized rabbits (18 cases) in ice-cold water for 10 min the colonic temperature fell from 38 to 30 C. By 2 h plasma TSH levels were again unchanged ($p > 0.05$) and plasma corticosterone levels had risen from 5.9 to 27.4 μg/100 ml ($p < 0.001$ 10 cases) or were unchanged ($p > 0.05$) but high in animals pretreated with stereotactically around the preoptic area provoked a decrease in hypothalamic temperature by 3 C (8 cases) while plasma TSH fell to an undetectable level adn corticosterone rose from 18.7 to 26.2 ($p < 0.05$) μg/100 ml. We conclude that in the rabbit the hypothalamus is refractory to cold stimulus possibly through a mechanism associated with the secretion of ACTH

Accelerated function of the pituitary thyroid axis during exposure to cold is believed to be due to activation of neural thermoreceptors (Uotila 1940 Andersson *et al* 1964 Kajihara *et al* 1972) or to augmented utilization of thyroid hormones in tissues or through the intestinal tract (Freinkel and Lewis 1957 Galton and Nisula 1969). Both these mechanisms can elicit secretion of pituitary TSH However measurements of increased blood TSH concentrations after exposure to cold have been successful only in the rat (Jobin and Samel 1964 Ducommun *et al* 1966). In adult human beings for example external cooling fails to evoke acute TSH release (Berg *et al* 1966 Odell *et al* 1967 Hershman *et al* 1970 Fisher and Odell 1971)

In experimental animals exposure to cold represents a stress stimulus which may modify the thyroid response to cold (Ducommun *et al* 1966 Leppäluoto 1972) The present investigation was therefore undertaken to measure the acute secretions of ACTH and TSH in the rabbit after exposure to various degrees of cold

Material and methods

1 *Animals and diet* 45 healthy adult male rabbits (2—2 5 kg) were fed on a pelleted diet (iodine concentration 0 6 mg/kg) and tap water ad lib The range of the temperature in the experimental and animal rooms was 20—24 C

2 *Assessments of the secretions of TSH and ACTH* The plasma TSH concentration was measured by the McKenzie bioassay (McKenzie 1958) In our hands this method detects 15 μU of bovine TSH which corresponds to a lower limit of detection of 30 μU/ml with an injection volume of 0 5 ml (Leppaluoto 1972) The secretion of ACTH was followed by estimation of plasma corticosterone concentration by a fluorometric method (Spencer Peet *et al* 1965)

3 *Experimental procedures* All the experiments wer begun at 11 a.m

A *Transfer studies* Nineteen rabbits were transferred to a room with a temperature of 10 C for 1—3 h Blood samples were drawn from ear veins at 0 1 and 3 h

B *Immersion studies* Ten rabbits were anesthetized intravenously with 5—10 ml of a solution contained 4 3 g chloral hydrate 1 0 g pentobarbital 2 1 g $MgSO_4$ propylene glycol 36 ml and ethanol 8 2 ml in 100 ml (Equitesin® Jen Sal USA) One hour later the anesthetized animals were immersed in ice-cold water for 10 minutes and then left at room temperature Before immersion the fur of the animals was moistened with detergent Colonic temperature was followed with a thermometer (Elema) Because of the greatly decreased skin circulation during this technique the ear vein was cannulated with a feeding tube AR—34R (Argyle U K) Blood was collected at 0 1 and 2 h after immersion 8 rabbits were given dexamethasone (Orgadrex® 5 mg Organon) s c 14 and 2 h before immersion and then treated as above

C *Hypothalamic cooling* Eight animals were anesthetized as before and 4 silver thermodes insulated with glass (1 mm thick) except for 0 5 mm at the tip were implanted stereotactically around the preoptic area The co ordinates according to Jokinen (1973) were AP+2 mm laterally 2 mm (left and right) depth 16—18 mm and AP—2 mm similarly The thermode was cemented to the skull during the experiment The upper end of the thermode was also free of insulation and situated in a small box above the skull The preoptic area was cooled by running ice cold water through the box For recording of preoptic temperature a needle thermometer 0 2 mm thick was implanted stereotactically between the thermodes The colonic temperature was followed as above

4 *Comparison of the effects of various treatments on serum corticosterone and TSH levels* A comparison between continuous variables (serum corticosterone levels and temperatures) was made by Student s t test (Snedecor 1956) The changes of serum TSH level before and after the treatment were compared by the sign test (Snedecor 1956) The values for p were regarded as significant when below 0 05 and highly significant when below 0 01

Results

Transfer studies on rabbits Before the transfer of 19 conscious rabbits to a cold room plasma TSH was detectable in 7 cases and after the transfer no consistent change ($p > 0.05$ sign test) in plasma TSH levels could be seen at 1 or 3 h (see Table I) The initial corticosterone level was 6 8 μg/100 ml and rose transiently after the transfer to 15 8 μg/100 ml ($p < 0.05$ t test) but fell again to the initial level at 3 h

Immersion studies on rabbits In anesthetized rabbits plasma TSH was detectable in 3 out of 10 cases before immersion During immersion of the rabbits in ice cold water the colonic temperature fell from 38 3 to 29 9° C ($p < 0.01$ t test) but again no consistent ($p > 0.05$ sign test) change in plasma TSH levels could be seen (Table II) The plasma corticosterone level rose from 5 9 to 27 4 μg/100 ml ($p < 0.01$ t test) by 2 h (Table II)

Immersion studies on rabbits pretreated with dexamethasone Before immersion plasma TSH was detectable in 5 out of 8 cases (see Table III) After immersion the colonic temperature fell from 38 6 to 30 8° C ($p < 0.01$ t test) and the plasma TSH again presented no uniform change ($p > 0.05$ sign test) (Table III) Plasma corticosterone levels also showed no alteration ($p > 0.05$ t test) in cold exposed

TABLE I The effect of transfer to a cold room on the plasma TSH and corticosterone levels in 19 conscious rabbits

	Rabbit no	Time after transfer in hours		
		0	1	3
Plasma TSH μU/ml	1	< 30	90	–
	2	125	< 30	–
	3	< 30	45	–
	4	70	80	–
	5	60	37	37
	6	< 30	–	< 30
	7	37	–	< 30
	8	45	–	< 30
	9	< 30	40	–
	10	40	< 30	–
	11	< 30	< 30	–
	12	< 30	< 30	–
	13	30	–	40
	14	< 30	–	37
	15	< 30	–	35
	16	< 30	–	< 30
	17	< 30	–	< 30
	18	< 30	–	< 30
	19	< 30	–	< 30
Plasma corticosterone μg/100 ml	1 – 19	6.8 ± 4.4	15.8 ± 5.2*	8.3 ± 3.8
Number of observations		19	9	11

Mean and ± S D are given
* = $p < 0.05$ (from the 0h value)
= $p < 0.01$
= $p < 0.001$
– = not measured
Note that the plasma TSH level increases within 1 h in rabbits no 1 3 4 and 9 and decreases in rabbits no 2 5 and 10 the change is not statistically significant ($p > 0.05$ sign test)

TABLE II The effect of cold immersion on the colonic temperature plasma TSH and corticosterone levels in 10 anesthetized rabbits

	Rabbit no	Time after immersion in hours		
		0	1	2
Colonic temperature °C	20 – 29	38.3 ± 1.1	29.9 ± 4.4	28.2 ± 5.6*
Plasma TSH μU/ml	20	30	30	–
	21	30	50	30
	22	56	30	30
	23	30	< 30	< 30
	24	56	56	–
	25	35	35	35
	26	30	37	30
	27	30	33	30
	28	30	30	–
	29	30	30	–
Plasma corticosterone μg/100 ml	20 – 29	5.8 ± 2.5	8.2 ± 2.9	27.4 ± 4.5*
Number of observations		10	10	6

See explanation in Table I

TABLE III The effect of cold immersion on the colonic temperature plasma TSH and corticosterone levels in eight anesthetized rabbits pretretaed with dexamethasone

	Rabbit no	Time after immersion in hours	
		0	1
Colonic temperature C	30–37	38 6±4 2	30 8±5 1**
Plasma TSH μU/ml	30	50	< 30
	31	< 30	< 30
	32	< 30	< 30
	33	< 30	67
	34	60	60
	35	270	< 30
	36	50	50
	37	45	< 30
Plasma corticosterone μg/100 ml	30–37	7 7±1 2	6 3±2 2
Number of observations		8	8

See explanations in Table I

rabbits (Table III) but were somewhat high in spite of pretreatment with dexamethasone

Hypothalamic cooling of anesthetized rabbits Before cooling the rectal and hypothalamic temperatures were identical but after central cooling for 15 min the hypothalamic temperature fell from 38 5 to 35 6° C ($p < 0\,01$ t test) and rose to 37 4 again when cooling ended Plasma TSH was detectable in 4 out of 8 cases before

TABLE IV The effect of hypothalamic cooling (15 min) on the hypothalamic and colonic temperatures and on the plasma TSH and corticosterone levels in eight anesthetized rabbits

	Rabbit no	Time after hypothalamic cooling in hours				
		0	1/4	1/2	1	2
Hypothalamic temperature C	38–45	38 5±1 0	35 6±0 8**	37 4±1 3	37 0±0 9	
Colonic temperature C	38–45	38 3±2 7	38 2±2 4	38 0±2 2	37 5±2 4	37 2±4 1
Number of observations		8	8	8	8	8
Plasma TSH μU/ml	38	45	—	< 30	< 30	—
	39	60	—	< 30	< 30	< 30
	40	67	—	< 30	< 30	< 30
	41	70	—	< 30	< 30	30
	42	30	—	< 30	< 30	30
	43	< 30	—	< 30	< 30	—
	44	30	—	< 30	—	—
	45	< 30	—	< 30	—	—
Plasma corticosterone μg/ml	38–45	18 7±6 0		24 3±5 1	26 2±4 1	19 9±5 7
Number of observations		8		8	6	4

See explanations in Table 1
¹ from 0 to 1/4 h

cooling but not after (Table IV) The plasma corticosterone level was clearly high before cooling (18 7 μg/100 ml) and still further increased after it ($p < 0.05$ t test Table IV)

Discussion

At the moment the plasma TSH concentration presents the most reliable indicator to the environmental stimuli affecting the thyroid gland In low ambient temperature the increase of plasma TSH level may be mediated by neural pathways or by increased peripheral utilization of thyroid hormones (Uotila 1940 Freinkel and Lewis 1957 Andersson *et al* 1964 Galton and Nisula 1969) It has been claimed that the rise in plasma TSH level must occur rapidly if it is mediated by neural mechanisms (Fischer and Odell 1971)

So far the plasma TSH level has been found to rise rapidly in a cold environment only in infants (Fischer and Odell 1971) and in one animal species the rat (Jobin and Samel 1964 Ducommon *et al* 1966) In this laboratory we showed that the transfer of the test animal to a cold room for 30 min provoked a rise in the plasma TSH level in the rat but not in the rabbit(Leppaluoto 1972 Lybeck *et al* 1973) In similar conditions in the rabbit however a massive (about 500 fold) increase in the plasma TSH level was found at three h by another worker (Bottari 1957) In the present study transfer of rabbits from 22° C to 10 C failed to evoke TSH release within 3 h and thus our finding strongly contrasts with that of Bottari (1957) The *in vitro* bioassay method used by Bottari (1957) was later challenged as non specific (Rerup and Melander 1965 Manley *et al* 1969) Hence it is more probable that acute exposure to cold fails to evoke TSH release in the rabbit

The present and earlier (Lybeck *et al* 1973) experiments from this laboratory show that during short term exposure to external cold there is an increase in the plasma corticosterone concentrations in both the rabbit and the rat but only the rat shows a rise in plasma TSH level This difference may be due to the fact that the secretion of TSH is inversely correlated with that of ACTH in the rabbit (Leppaluoto 1972) but not in the rat (Ducommun *et al* 1966)

The procedures used here in connection with hypothalamic cooling or immersion were unexpectedly stressful In this study plasma corticosterone levels after the cold stimulus were about twice as high as found earlier after the stress caused by handling (sampling stress) or administration of maximal doses of ACTH (Leppaluoto 1972) In view of the above discussed dependence of TSH secretion on ACTH secretion it was not unexpected that the plasma TSH level during immersion or hypothalamic cooling tended to decrease (Table IV) It has similarly been found that even in unanesthetized unrestrained rats hypothalamic cooling depresses the plasma TSH level possibly owing to non specific nociceptive stimuli associated with this procedure (Jobin *et al* 1971) However in the classical studies of Andersson *et al* (1964) hypothalmic cooling in conscious goats was effective enough to produce increased secretion of $PB^{131}I$ The lack of such a reflex in the rabbit suggests that i

this species the hypothalamus is refractory to the cold stimulus through a mechanism that might be associated with the secretion of ACTH

The support of the Yrjo Jahnsson foundation is gratefully acknowledged

References

ANDERSSON B C C GALE and B HOKFELT Studies of the Interaction between Neural and Hormonal Mechanismus in the Regulation of Body Temperature In *Major Problems in Neuroendocrinology* Ed E BAJUSZ and G JASMIN Basel/ New York S Karger 1964 42—61

BERG G R R D UTIGER D S SCHALCH and S REICHLIN Effect of central cooling in man on pituitary thyroid function and growth hormone secretion *J appl Physiol* 1966 *21* 1791—1794

BOTTARI P M The concentration of thyrotrophic hormone in the blood of the rabbit under different expenmetnal conditions *Ciba Found Coll Endocrinology* 1957 *11* 52—69

DUCOMMUN P E SAKIZ and R GUILLEMIN Dissociation of the acute secretions of thyrotropin and adrenocorticotropin *Amer J Physiol* 1966 *210* 1257—1259

FISHER A D and W D ODELL Effect of Cold on TSH Secretion in Man *J clin Endocr* 1971 *33* 859—862

FREINKEL N and D LEVIS The effect of lowered environmental temperature on the peripheral metabolism of labelled thyroxine in the sheep *J Physiol* (Lond) 1957 *135* 288—300

GALTON V A and B C NISULA Thyroxine Metabolism and Thyroid Function in the Cold Adapted Rat *Endocrinology* 1969 *85* 79—86

HERSHMAN J M D G READ A L BAILEY V D NORMAN and T B GIBSON Effect of Cold Exposure on Serum Thyrotropin *J clin Endocr* 1970 *30* 430—434

JOBIN M and M SAMEL Pituitary thyroid response to cold exposure in the rat *Proc Can Fed Biol Soc* 1964 *7* 23

JOBIN M E ENDROCZI S HONTELA and C FORTIER Effect of hypothalamic cooling on the behaviour and the pituitary thyroid activity of the rat *J Physiol* (Paris) 1971 *63* 309—311

JOKINEN Y The activation mechanism of hypothalamic temperature sensitive neurones in the rabbit 1973 In press

KAJIHARA A T ONAYA T YAMADA Y TAKEMURA and M KOTANI Further Studies on the Acutes Stimulatory Effect of Cold on Thyroid Activity and Its Mechanism *Endocrinology* 1972 *90* 538—544

LEPPALUOTO J Blood bioassayable thyrotropin and corticosteroid levels during various physiological and stress conditions in the rabbit *Acta endocr* (Kbh) 1972 *165* Suppl 1—38

LYBECK H J LEPPALUOTO and P VIRKKUNEN The effect of pertinent and non pertinent stimuli on the secretions of thyrotrophin and corticotrophin *Int J Neurosci* 1973 *5* 47

MANLEY S W J R BOURKE and R W HAWKER An in Vitro Bioassay for Thyrotropin (TSH) *Endocrinology* 1969 *84* 1286—1291

MCKENZIE J M The bioassay of thyrotropin in serum *Endocrinology* 1958 *63* 372—382

ODELL W J WILBER and R UTIGER Studies of Thyrotropin Physiology by Means of Radioimmunoassay *Recent Progr Hormone Res* 1967 *23* 47—85

RERUP C and A MELANDER On the bioassay of thyrotropin in plasma *Acta endocr* (Kbh) 1965 *50* 177—194

SNEDECOR G W In *Statistical Methods* Ed the Iowa State College Press Iowa 1956 117

SPENCER PEET J J R DALY and V SMITH A simple method for improving the specificity of the fluorometric determination of adrenal corticosteroids in human plasma *J Endocr* 1965 *31* 235—244

UOTILA U On the role of the pituitary stalk in the regulation of the anterior pituitary with special reference to the thyrotropic hormone *Endocrinology* 1940 *25* 605—614

Acta physiol scand 1973 89 429—435

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Effects of Extrinsic Denervation on the Fluorescence of Monoamines in the Small Intestine of the Cat

By

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Abstract

AHLMAN H L ENERBÄCK J KEWENTER and B STORM *Effects of extrinsic denervation on the fluorescence of monoamines in the small intestine of the cat* Acta physiol scand 1973 *89* 429—435

The effects of section of the nerves supplying the small intestine in the cat were studied *in vivo* at different times after the operation Before denervation biopsies from the jejunum and ileum were studied by a fluorescence method in order to observe the adrenergic innervation and the presence of 5 hydroxytryptamine (5 HT) In addition to the adrenergic fibres terminating around the ganglion cells of the intestinal nervous plexuses other adrenergic fibres were seen in close connection with the muscle fibres in both muscular layers The only location of 5 HT seemed to be in the enterochromaffin cells of the mucosa After nerve section the completeness of the denervation was established in an acute experiment by means of electrical stimulation of the vagal and splanchnic nerves while registering the motility of a jejunal and an ileal loop Afterwards the small intestinal wall was studied with the same histochemical fluorescence method No changes were found in the bowel habits or in the general state of nutrition of the animals The intestinal adrenergic innervation completely disappeared after denervation The adrenergic innervation does not seem to be necessary for adequate peristaltic activity in the small intestine 5-HT did not disappear after denervation but was found unchanged in the enterochromaffin cells

The motility of the small intestine is controlled by a complex neuro myo endocrine system Thus far little is known about the functional significance of the two nervous components the intrinsic and the extrinsic systems The intrinsic nervous system is probably involved in the propagation of intestinal content (Kosterlitz 1967 Hukuhara *et al* 1959) but there have been very few *in vivo* studies dealing with this problem The extrinsic nervous system has been studied in many acute experiments and it can be concluded that the parasympathetic nervous system induces a momentary contraction of the gut and the adrenergic system an inhibition of the lower part of the small intestine and the colon (Hulten 1969 Kewenter 1965) Information about the extrinsic nervous control of the intestinal sphincter regions is sparse T' aim of the present investigation was to study the effect of chronic extr

tion of the small intestine in cats and its influence upon the amounts and distribution of noradrenaline and 5 HT in the intestinal wall as studied by fluorescence microscopy

Methods

12 cats of either sex were used After induction of anesthesia with ether 30 mg/kg of Mebumal sodium was injected i p The abdomen was opened by a midline incision The nerve trunks along the superior mesenteric artery were cautiously isolated and cut. The mesentery was then divided from the point where the nerve trunks were cut proximally to the flexura duodenojejunalis and distally to the ileo-caecal sphincter All vessels in these two incisions were ligated Great care was taken to cut all tissue close to the gut The pancreas liver and gallbladder were not denervated Thus the small intestine was denervated and only connected to the rest of the body via the superior mesenteric artery the mesenteric vein and the intestine proximally and distally The abdominal wall was closed and the animals were allowed to recover After the denervation the animals were checked with respect to body weight food intake bowel habit and the general condition

After one month (9 animals) two months (2 animals) and one and a half year (1 animal) the completeness of the denervation was tested in an acute experiment For this purpose the animals were anesthetized with chloralose i v a tracheal cannula was inserted and the abdomen opened A description of the technique has been presented previously (Kewenter 1965) Briefly a proximal and a distal loop within the denervated part of the small intestine were isolated with an intact vascular supply The proximal jejunal loop was divided from the duodenum between ligatures below the flexura duodeno jejunalis and the distal ileal loop was divided distally about 5 cm from the ileo-caecal sphincter Each loop was about 8 to 10 cm long The intestinal contents were removed and the motility in each loop was measured by means of a volume recording system

The vagal and splanchnic nerves were cautiously dissected free in the neck and abdomen respectively The nerves were cut proximally and the distal end of each nerve was placed on a coiled silver electrode The electrodes were connected to a Grass Stimulator S 4 The nerves could be stimulated separately or simultaneously Square wave stimulations (frequency 2—16 Hz strength 0 02—20 V duration 0 05—10 ms) were used

To eliminate catecholamine secretion from the adrenal glands on splanchnic nerve stimulation the vessels of both glands were ligated

The distribution of monoamines was studied with the formaldehyde condensation method described by Falck and Hillarp (1962) From 7 of the operated cats representing all three periods of observation biopsies were taken from different parts of the bowel before and one month after the denervation 2 normal cats served as controls The biopsies were quenched in propane over liquid nitrogen freeze dried and treated with formaldehyde gas equilibrated at 70 relative humidity at 80 C for 1 h (Hamberger *et al* 1965) The specimens were embedded in paraffin sectioned and mounted in a mixture of xylene and Entellan® (50/50)

In some sections the specificity of the fluorescence was checked with borohydride treatment (Corrodi et al 1964)

For fluorescence microscopy a Zeiss fluorescence microscope equipped for activation with transmitted light from an Osram HBO 200 mercury burner was used A Schott BG 12 filter combined with a barrier filter with the wedge at 500 nm was used With this filter combination which has been used extensively 5 HT and certain other tryptamine derivatives show a yellow fluorescence and catecholamines appear yellow green For critical evaluation of the colour of the fluorophores a Leitz Dialux microscope equipped for transmitted light illumination with a mercury burner of high intensity (Osram HBO 50) was used The activation maxima of the fluorophores (410 nm for both trypamine and catecholamines) were isolated with a combination of a Schott BG 3 glass filter and an interference filter (Schott 407 12) (Enerbäck and Häggendal 19?0) The fluorescent light was filtered through a barrier filter with the wedge at 430 nm With this filter combination tryptamine appear bright yellow and catecholamines blue or blue green (Ploem 19?9

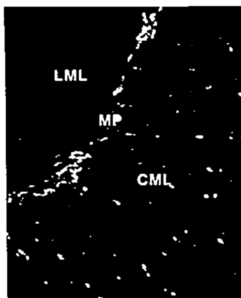


Fig 1 Section from the ileum of a normal cat LML = longitudinal muscle layer CML = circular muscle layer MP = myenteric plexus Strongly fluorescent adrenergic nerve fibres surrounding the ganglion cells in MP Radiating transverse fibres (parallel to the plane of section) and longitudinal (transversely cut) nerve fibres in CML Sparse adrenergic innervation of LML

Results

All animals survived the operation without difficulty During the observation time the animals had the same intake of fluid and food as a control group The body weight did not change during the period of observation We could not detect any changes in the animals bowel habits

Electrical stimulation of the splanchnic nerve did not induce any inhibitory motor responses in the jejunum or the ileum *i e* there was no increase in the volume of the loops although the stimulation parameters were varied over a wide range Vagal stimulation induced a clear cut but small motor response in the jejunal loop in 4 of the 12 cats In 2 of these animals small motor responses were also obtained in the ileum No differences were observed between these animals and those in whom vagal nerve stimulation did not induce any motor response All postoperative histochemical studies were performed in the completely denervated cats Parasympathetic denervation of the small intestine seems to be difficult to perform In a previous series of acute experiments it was found necessary in a small number of animals to put local anesthetic around the superior me enteric artery in order to block all parasympathetic fibres although all nerve fibres around this artery were macroscopically cut

As described by previous investigators (Norberg 1964 Jacobowitz 1965) the jejunal and ileal walls were found to contain numerous perivascular adrenerg

cosities showing a strong blue or blue green fluorescence Numerous adrenergic nerve fibres of small diameter were seen in the connective tissue separating the circular and longitudinal muscle layers These fibres formed networks around the ganglion cells of the myenteric plexus, which were identified by simultaneous phase contrast and fluorescence microscopy Similar small fibres though less numerous were also seen in the submucous plexus The circular muscle layer was found to contain a richer innervation than the longitudinal layer The circular layer contained numerous fibres parallel to the muscle bundles and fewer fibres running perpendicular to the muscle bundles (Fig 1) The longitudinal layer contained few fibres most of them running parallel to the muscle bundles In the mucous coat no adrenergic fibres were seen but numerous bright yellow fluorescent enterochromaffin cells were easily recognized They had a triangular shape with the base directed towards the lumen of the glands No yellow fluorescence of the tryptamine type could be observed in other locations in the intestine In some specimens yellow or orange fluorescence was observed in the connective tissue separating the longitudinal and circular muscle layers The fluorescent material had a granulated structure and unlike the fluorescence of enterochromaffin cells was very little affected by prolonged ultra violet illumination Continuous illumination for up to 10 min resulted in a very pronounced fading of the fluorescence of enterochromaffin cells and adrenergic nerve fibres but did not noticeably decrease the fluorescence of this material Furthermore this fluorescence was not abolished by treatment of the sections with sodium borohydride

In the sections from denervated portions of the jejunum and ileum there was a complete lack of fluorescent adrenergic nerve fibres (Fig 2) Enterochromaffin cells of the mucosa appeared unchanged both in number and in fluorescence intensity as judged by eye All 7 cats used in the experiment thus lacked adrenergic fluorescence No signs of regrowth of autonomic nerve fibres such as sprouting were seen

Discussion

The fluorescence method of Falck and Hillarp provides a very specific and sensitive tool for the localization of monoamines in tissues (Falck 1962) 5 hydroxytryptamine and its precursor 5 hydroxytryptophane show a yellow fluorescence on condensation with formaldehyde and the catecholamines (mainly dopamine and noradrenaline) form fluorescent componds which appear blue or green depending upon the conditions of fluorescence microscopy The fluorescence microscopical technique used in the present investigation provides optimal conditions for distinguishing between the yellow tryptamine fluorescence and the blue or blue-green catecholamine fluorescence The distribution of adrenergic nerve fibres in the intestine found in the present investigation confirms the recent observations of Silva et al (1971) on the ileum of the cat and of Costa and Gabella (1971) on the small intestine of the rabbit A rich adrenergic innervation was found around the ganglion cells especially in the myenteric plexus Numerous smaller nerve fibres were also

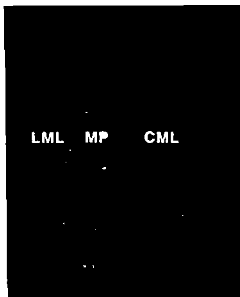


Fig 2 Section from a denervated segment of the ileum Symbols as in Fig 1 Note absence of adrenergic innervation

found especially in the circular mu cle coat No intrinsic adrenergic neurones were found in the myenteric plexus as described by Furness and Costa (1971) in the proximal colon of the guinea pig

It has been suggested that the inhibitory effect of these fibres which are found around the intramural ganglion cells in the cat is mediated by the postganglionic parasympathetic ganglion cells (Norberg 1964 Jacobowitz 1965 Kewenter 1965)

However Hollands and Vanov (1965) working on the rat duodenum and rabbit colon found a great number of adrenergic fibres adjacent to the smooth muscle cells which they thought to be directly innervated by these fibres Our similar finding sug gest that two types of inhibitory mechanisms exist one with a direct action on the smooth muscle layers one with an effect on the intramural parasympathetic ganglion cells Such an innervation has been postulated in the guinea pig ileum by Kosterlitz and Watt (1965) who suggested that the former inhibition was mediated via β and the latter inhibition via α receptors Whether each of the e is activated under dif ferent circumstances is not known

In contrast to other reports no fluorescence of the tryptamine type could be demonstrated near the myenteric plexus 5 HT was found in the myenteric plexus by Tafuri and Raick (1964) in a histochemical study in the guinea pig By radio-autography in mice Gershon and Ross (1966 found an uptake and rapid turnover of tritium labelled 5 HT in the terminal axons of the myenteric plexus This uptake is not nonspecific shown by Gershon and Altman (1971) In the cat we could not confirm the presence of 5 HT in any other location than in the enterochromaffin cells In the present experiments we can not exclude that the 5 HT conten myenteric plexus is too low for histochemical demonstration as discussed



Fig 3 Numerous enterochromaffin cells in a denervated segment of the ileum. Fluorescence unaffected by denervation

son and Gershon (1971) This possibility will however, be examined in another series of experiments The granulated yellow or orange coloured material which was occasionally observed in the connective tissue separating the muscle layers differed from the tryptamine fluorescence of enterochromaffin cells in several respects The colour of the fluorescence was different and the fluorescence lacked the pronounced fading resulting from continuous illumination typical for the 5 HT fluorophor The fluorescence of this granulated material was not abolished by sodium borohydride. These findings indicate that the fluorescence of the granulated material is not due to a tryptamine derivative but due to an autofluorescent compound possibly a lipofuscin like pigment

In the denervated portions of the intestine there was a complete lack of adrenergic fluorescent nerve fibres The histochemical fluorescence method is thus a good indicator of the level as well as the completeness of the denervation The present results confirm previous findings that the adrenergic fibres in the intestinal wall do not originate locally but from cell bodies outside the gut probably localized in the prevertebral sympathetic ganglia (*Langley 1922 Norberg 1964*) The findings that chronic section of all nerves supplying the small intestine depletes it completely of noradrenaline indicate that the presence of noradrenaline in the intestinal wall is not necessary for maintaining its propulsive activity The strong fluorescence of enterochromaffin cells due to 5 HT seemed quite unaffected by the denervation

The functional significance of the extrinsic adrenergic nerve fibres to the small intestine is unclear but it seems reasonable to believe that they can inhibit the peristaltic activity as has been shown in the isolated colon of the rabbit (Lee 1960) When this mechanism is activated is not known but it is possible that postoperative paralytic ileus for example is mediated via this mechanism This is further indicated by the fact that it is possible to influence this functional ileus by administration of an α blocking agent, which is without effect on the locally mediated propulsive activity (Petri *et al* 1971) Furthermore it has been shown by Lindqvist (1971) in experi

ments in rats that the concentration of catecholamines in the intestinal wall is elevated immediately after deposition of a retroperitoneal irritant He concluded that the intestinal paralysis provoked in this way was mediated via the sympathetic nervous system These experiments indicate that the extrinsic adrenergic nerves are involved in the inhibitory regulation of propulsive motor activity

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References

CORRODI H N A HILLARP and G JONSSON Fluorescence methods for histochemical demonstration of monoamines 3 Sodium borohydride reduction of the fluorescent compounds as a specificity test. *J Histochem Cytochem* 1964 *12* 582

COSTA M and G GABELLA Adrenergic innervation of the alimentary canal Z *Zellforsch* 19/1 *12?* 3ɔ7—377

ENERBACK L. and J HAGGENDAL Uptake of dopamine by rat mast cells in vivo *Pathol Europ* 19/0 *5* 401—408

FALCK B Observations on the possibilities of the cellular localization of monoamines by a fluorescence method *Acta physiol scand* 196? *56* Suppl 197

FALCK, B N A HILLARP G THIEME and A TORP Fluorescence of catecholamines and related compounds condensed with formaldehyde *J Histochem Cytochem* 196? *10* 348

FURNESS J B and M COSTA Morphology and distribution of intrinsic adrenergic neurones in the proximal colon of the guinea pig *Z Zellforsch* 1971 *1?0* 346—363

GERSHON M D and R F ALTMAN An analysis of the uptake of ɔ-HT by the myenteric plexus of the small intestine of the guinea pig *J Pharmacol exp Ther* 1971 *179* ?9—41

GERSHON M D and L. L. ROSS Location of sites of 5 HT storage and metabolism by radioautography *J Physiol* (Lond) 1966 *186* 477—492

HAMBERGER B T MALMFORS and CH SACHS Standardization of paraformaldehyde and of certain procedures for the histochemical demonstration of catecholamines *J H stochem Cytochem* 1965 *13* 147

HOLLANDS B and S VANOV Localization of catecholamines in visceral organs and ganglia of the rat guinea pig and rabb t *Brit J Pharmacol* 1965 *25* 307—316

HUKUHARA T S NAKAYAMA and T SUMI The role of the intrinsic mucosal reflex in the fluid transport through the denervated intestinal loop *Jap J Physiol* 1959 *9* 406—408

HULTÉN L. Extrinsic nervous control of colonic Motility and Blood flow An experimental study in the cat *Acta physiol scand* 1969 Suppl 335

JACOBOWITZ D Histochemical stud es of the autonomic innervation of the gut *J Ph rmacol exp Ther* 1965 *149* *3* 3ɔ8—364

KEWENTER, J The vagal control of the jejunal and ileal motility and blood flow *Acta physiol scand* 1965 Suppl 257 1—68

KOSTERLITZ H W Intrinsic intestinal eflexes *Amer J dig D* 1967 *1?* (N S) 245—254

KOSTERLITZ H W and A. J WATT Adrenergic recepto s in the guinea pig ileum *J Phy iol* (Lond) 1965 *177* 11—12 P

LANGLEY J N Connexions of the enteric nerve cells *J Physiol* (Lond) 19?2 *56* 39 P

LEE C Y The effect of stimulation of extrinsic n rves on peristalsi and on the release of 5 HT i the large intestine of the guinea pig ard of the abbit *J Phy ol* (Lond) 1960 *15?* 40ɔ—418

LI DQVIST B Role of the s mpathet n ous system n retroperitoneally induced intest nal paralysis *Acta chi scand* 19 1 13 459—46?

NORBERG K A Adrenergic innervat on of the intestinal all stud ed by fluoresce ce mic oscop *Int J Neu ophatma ol* 1964 *3* 379—38?

PETRI G J SZENOHRADSKY adn M PORS ASZ-GIBISZER Sympatholytic treatment of paralytic" ileus *Su gery* 1971 *70* 359—ɔ67

PLOEM J S A new mi croscopi method for the visualization of blue formaldehyde induced catecholamine fluorescence *Arch int Pha macodyn* 1969 *18?* 421—4?4

ROBINSON R G and M D GERSHON Synthesis and uptake of 5 HT by the myent ric ple us of the guinea pig ileum A histoche nical study *J Pha macol e p Th* 1971 *178* 311—3?4

SILVA D G ROSS and L. W OSBORNE Adrenergic innervat on of the ileum of the cat *Am J Physiol* 1971 *??0* 347—35?

TAFURI W L. and A RAICK Presence of 5-HT in the intramural nervous syst pig's intestine *Z Naturfo schg* 1964 *19 b* ?ɔ—1128

Acta physiol. scand. 1973. 89. 436—448

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Ionic Fluxes in the Rat Portal Vein and the Applicability of the Goldman Equation in Predicting the Membrane Potential from Flux Data

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Abstract

WAHLSTRÖM, B. A. *Ionic fluxes in the rat portal vein and the applicability of the Goldman equation in predicting the membrane potential from flux data.* Acta physiol. scand. 1973. *89*. 436—448.

The sodium, potassium and chloride effluxes from the rat portal vein were measured using radioactive tracer ions. It was found that the sodium efflux attributable to an intracellular compartment was very slow with a rate constant of 4.66×10^{-4} s^{-1} and the corresponding intracellular sodium only 3.0 mmol/kg wetweight. The intracellular potassium exchanged with a rate constant of 2.15×10^{-4} s^{-1} and contained 45.5 mmol/kg wetweight. The intracellular chloride content was 14.7 mmol/kg wetweight, exchanging with a rate constant of 10.55×10^{-4} s^{-1}. From these data the permeabilities of Na, K and Cl were calculated to 0.130, 3.81 and 3.11×10^{-8} cm s^{-1} respectively. The relative permeabilities $P_K : P_{Na} : P_{Cl}$ were 1 : 0.034 : 0.816 and from these values the Goldman equation predicted a membrane potential of −42.3 mV. The actual membrane potential measured by glass microelectrodes was −45 mV. It was concluded that the membrane potential in the rat portal vein in normal solution can be predicted from the permeabilities and distribution of K and Cl, while Na plays an insignificant part.

Ionic fluxes and ionic distribution between intracellular and extracellular spaces can be used to calculate ion permeabilities if no net ion movements occur. Goldman (1943) formulated an equation which relates the permeabilities and the intracellular and extracellular concentrations of sodium, potassium and chloride to the membrane potential. Hodgkin (1951) applied this equation to flux data from the squid axon and found a very good agreement between the calculated and measured membrane potential. The equation was also successfully applied to frog skeletal muscle (Hodgkin and Horowicz 1959). In smooth muscle, however, predictions of the membrane potential from flux data have been less accurate. Casteels (1969) calculated the resting potential in the smooth muscle taenia coli of the guinea pig by the Goldman equation and predicted a membrane potential of −37 mV, as compared to the

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easured value of -57 mV He proposed a sodium pump supposedly electrogenic o explain the difference Casteels and Kuriyama (1966) studied the ion content of enia coli at different external K concentrations and concluded that the membrane otential was mainly a potassium dependent potential but that chloride was non assively distributed and might contribute to the membrane potential Ohashi 1970) gave further evidence for the importance of chloride in membrane con uctance However, the chloride permeability is of interest mainly if the equilibrium otential for chloride is very different from the membrane potential since in that ase changes in P_{Cl} may affect the resting potential and thereby the spontaneous ctivity of the tissue This study was undertaken to investigate the effluxes of sodium otassium and chloride and the distribution of these ions in normal solution and to pply the Goldman equation to the data In a concomitant paper the effects of nor drenaline on the effluxes and ion distribution were investigated Some results have een presented in preliminary form to the Physiological Society Meeting in Cam ridge July 1972 (Wahlström 1972)

Methods

Male white Wistar rats weighing 200—300 g were used They were killed by a blow over the neck and bled out. The portal vein was dissected and blotted on filter paper the freshweight (W_f) determined on a torsion balance and the muscle cut longitudinally and mounted on a tungsten wire holder The tissue was stretched to *in situ* length and fixed

Ion distribution and extracellular space experiments Muscles mounted on holders were in cubated in normal physiological salt solution PSS (composition see below) for a minimum of 2 h before beginning the experiment In some experiments ^{14}C sorbitol or ^{60}CoEDTA was added for 10 min at the end of the incubation period for extracellular space determinations (*ECS*) In these experiments a suitable amount of non active carrier was present in the PSS to avoid errors arising from adsorption of the marker on the tissue After the experiments test and control tissues were blotted on filter paper and the wetweight (W_w) determined. Each tissue was then transferred to a test tube containing 0 5 or 1 0 ml 2 mM $AgNO_3$ in 30 % H_2O_2 (100 vol 10) and digested overnight at 90 C When ^{14}C sorbitol was used the tissues were left for 24 h in 1 ml bidistilled H_2O and an aliquot of 0 1 or 0 2 ml taken for ^{14}C counting before the digestion mixture was added

Determination of ion content The digestion mixture was evaporated to dryness and 4 ml diluting fluid added The diluting fluid was a 1 M HNO_3 solution containing 10 mM L and 18 5 mM La^{+++} Na K Ca Mg and Ag were then determined directly on a Pye Unicam Atomic Absorption Spectrophotometer Cl was determined from the amount of AgCl formed *i e* the difference between the total amount of Ag added to each tube and the amount of Ag remaining after digestion as determined by absorption spectrometry

Efflux experiments Muscles mounted on holders were incubated in PSS containing either ^{24}Na ^{42}K or ^{36}Cl the solution otherwise being identical with a non radioactive solution The loading period was 90 min for ^{36}Cl and 3 h for ^{24}Na and ^{42}K The muscles were then trans ferred to a beaker containing inactive solution rapidly shaken for 5 s blotted and mounted in a constant flow chamber for measuring ion efflux (Brading 1967) The effluent was col lected in test tubes or scintillation vials every min or every other min After each wash [illegible] muscle was counted and either reloaded and a second washout performed or removed [illegible] weighed and digested as described above

^{24}Na ^{42}K and Cl counting The effluent from each tube was counted in a well [illegible] counter (EKCO) under conditions of constant geometry Muscles were counted [illegible] at the end of the washout The activity remaining in the tissue was added [illegible] each tube in reverse order giving at each point during the washout the total [illegible] tissue The rate of flux was obtained by dividing the activity coming out from the [illegible] given time by the total activity in the tissue at that time

TABLE I

	Na	K	Cl
Total ion content mmol/kg W_w	85 6±2 7 (14)	43 5±1 5 (14)	96 4±6 7 (14)
Extracellular space ml/kg W_w	572±25	572±75	572±25
Dryweight/wetweight %	21 0±0 9	21 0±0 9	21 0±0 9
Intracellular ion content mmol/kg W_w	*7 0*	*40 1*	*19 7*
Cell water concentration mM	32	182	90
Equilibrium potential mV	+39	−91	−11

^{36}Cl counting To the effluent in each vial was added 10 ml of Bray s mixture The vials were counted in a Beckman scintillation counter using a wide gate (0—∞) The muscle activity was extracted for 24 h in 1 ml bidistilled H_2O and an aliquot of 0 2 or 0 5 ml taken After efflux and extraction the mu cles in all efflux experiments were digested and the ion content determined as described above

Graphic and numerical procedures All values of ion content and extracellular space are expressed as mmol and ml per kg W_w respectively The data from ^{24}Na ^{42}K and ^{36}Cl counting were corrected for background decay and collection time and expressed as counts per s (cps) and plotted on a logarithmic scale against time The rate of flux was expressed as cps per min and plotted on a linear scale against time

Mean values are given with S E Differences between means were tested by Student s t test and considered to be significant if $p < 0.05$ The number of observations are given in brackets

Efflux curves were treated as multicomponent exponentials The late linear phase was extrapolated to zero time and subtracted from the total curve and th difference replotted This procedure gave two components for the ^{42}K efflux curve whi e the ^{24}Na and ^{36}Cl efflux curves could not be described by less than three exponentials

Solutions and chemicals The normal PSS had the following composition in mM Na 137 5 K 6 0 Ca^{++} 2 5 Mg^{++} 1 2 Cl 134 1 HCO_3 15 5 H_2PO_4 1 2 Glucose 11 5 K high solutions were prepared by substituting all NaCl by KCl giving a K concentration of 128 mM Solutions with 12 24 48 and 96 mM K were made by mixing appropriate amounts of normal PSS with K high PSS Ca free and Ca high PSS were made by substituting $CaCl_2$ for NaCl on an equimolar basis All solutions were aerated by 97 % O_2/3 % CO_2 and pH was 7 4 The following isotopes were supplied by the Radiochemical Centre Amersham England $^{24}NaCl$ isotonic solution ^{42}KCl isotonic solution $Na^{36}Cl$ isotonic solution ^{14}C sorbitol (U) and $^{60}CoCl$ The final activity in the loading solution varied between 4 and 50 μCi/ml depending on the isotope used

Results

Ion content and extracellular space in normal PSS When pieces of rat portal vein are excised from the body they rapidly lose potassium and gain sodium but during incubation in warm oxygenated PSS they gradually recover and after 2 to 3 h incubation the tissue content is almost back to what it was in the freshly dissected muscle and can be maintained at this level for many hours (Wahlstrom *1971*) Table I shows the results from an experiment in which pieces of portal vein were incubated for 3 h in normal PSS The extracellular space was determined by adding ^{60}CoEDTA as a marker during the last 10 min of incubation The Table shows that the calculated $[Na]_i$ is 32 mM and $[K]_i$ is 182 mM These values agree well with earlier data from the rat portal vein (Haljamae *et al* 1970 Wahlstrom 1971) The chloride content is quite high and the calculated $[Cl]_i$ much too high to be explained by a passive distribution When single cells were penetrated *by microelectrodes* it was found that the average membrane potential was −45±4 mV (10)



Fig 1 Spontaneous electrical activity of the rat portal vein recorded intracellularly by glass microelectrodes The upper continuous line shows the zero potential. Time and voltage calibrations are given by the horizontal and vertical bars Note that most of the action potentials overshoot the zero line by a few millivolts

(Fig 1) This value is 46 mV more positive than the potassium equilibrium potential E_K, and 34 mV more negative than E_{Cl} (see Table I) It seemed likely therefore that in this tissue either the sodium permeability P_{Na} was relatively high or that chloride played an important part in determining the level of the resting potential or a combination of these two factors was operating

Ion content in K high PSS and in the presence of ouabain If some or all of the tissue chloride moves passively across the membrane according to a Donnan equilibrium changes in the external potassium concentration ought to affect the chloride content of the portal vein When muscles were incubated for 3 h in K high PSS and the chloride and potassium contents determined there was a significant increase in the chloride content from 93 6 ± 1 4 mmol/kg W_w to 111 8 ± 2 6 mmol/kg W_w (6) ($p < 0\ 005$) The W_w/W_t ratio increased as well from 84 8 ± 2 8 % in normal PSS to 108 8 ± 4 8 % ($p < 0\ 01$) in K high PSS which shows that the tissue swell as predicted The potassium content was 128 1 ± 3 0 mmol/kg W_w The results suggest that in these rather extreme conditions the tissue to some extent obeys a Donnan equilibrium

On the other hand if chloride is actively transported into the cell against an electrochemical gradient by a pump mechanism inhibition of the pump ought to establish a passive equilibrium distribution When muscles were incubated for 1 h in a normal PSS containing 10 μM ouabain the potassium content fell from 49 7 ± 4.5 mmol/kg W_w to 24 8 ± 3 1 mmol/kg W_w The chloride content however did not change but was 90 5 ± 5.3 mmol/kg W_w in normal PSS and 87 8 ± 8 4 mmol/kg W_w in the ouabain treated muscles Thus although in K high PSS the tissue chloride partly obeys a Donnan equilibrium in normal PSS it probably does not.

^{24}Na efflux in normal PSS The efflux of ^{24}Na from a single muscle is shown in Fig 2 The curve follows a complex pattern and does not settle down to a single exponential until roughly 15 min after the start of the washout. The rate of flux (open circles in Fig 2) shows the same curvature for the first 10 min but is then more or less constant This complexity meant that the curves had to be further and the simplest way was to assume that we had to deal with a exponential efflux and to subtract the late linear phase to get at the ponents Such a treatment is shown in Fig 3 which is a mean curve for six

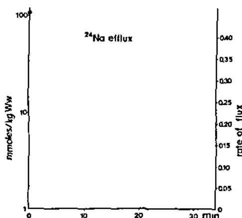


Fig 2

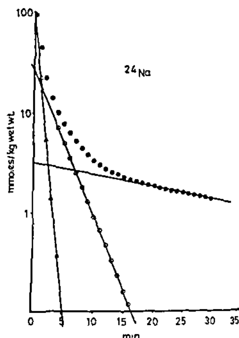


Fig 3

Fig 2 ^{24}Na efflux and rate of flux in normal PSS Filled circles Radioactivity in the tissue Open circles Rate of flux defined as the ratio between the amount of radioactivity (ΔR) leaving the tissue between time t and $t+\Delta t$ and the average total radioactivity ($\bar{R}\Delta t$) in the tissue during that interval Abscissa Time in min Ordinate left Radioactive sodium in the tissue in mmol/kg W_w right Rate of flux in arbitrary units The left scale is logarithmic while the right is linear

Fig 3 Analysis of ^{24}Na efflux curve The points representing radioactivity in the tissue in mmol/kg W_w (filled circles) can be considered to fall on a straight line after 15 min The middle component of the curve (open circles) was obtained by subtracting the extrapolated line for the slow component from the experimental values The values of this second *component* fell on a straight line after about 5 min and subtraction of this line from the earlier values gives the third and fastest component (open triangles) the values for which all fell on a straight line The slopes of the three lines represent the rate constants k_1 k_2 and k_3 for the three components A B and C obtained by extrapolation of the lines to zero time The efflux curve can thus be described by the equation ^{24}Na mmol/kg $W_w = A \times e^{-k_1 t} + B \times e^{-k_2 t} + C \times e^{-k_3 t}$ The curve shown in this figure is a mean curve for 6 muscles See also Text Abscissa *Time in* min Ordinate Tissue radioactivity in mmol/kg W

It is evident from the figure that three components were sufficient to describe the ^{24}Na efflux from the rat portal vein The mean rate constants for the three components were $k_1 = 2\ 39 \pm 0\ 64 \times 10$ s^{-1} $k_2 =$ [illegible] $4 \pm 0\ 65 \times 10^{-3}$ s^{-1} and $k_3 = 4\ 66 \pm 1\ 43 \times 10^{-4}$ s^{-1} The corresponding compartments were $A = 97\ 1 \pm 7\ 9$ mmol/kg W *$B = 24\ 2 \pm 8\ 2$ mmol/kg W_w and $C = 3\ 20 \pm 0\ 64$ mmol/kg W_w In the following* only mean curves will be shown Whether the first two compartments correspond to biophysical realities is another matter which will be discussed later

^{36}Cl efflux in normal PSS The washout curve for ^{36}Cl follows a pattern similar to that of ^{24}Na Fig 4 shows the mean efflux curve for 6 muscles in *normal* PSS

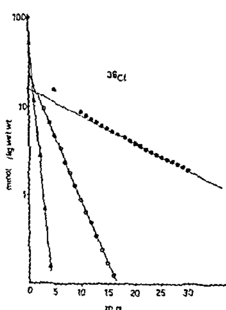


Fig 4

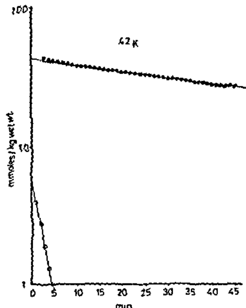


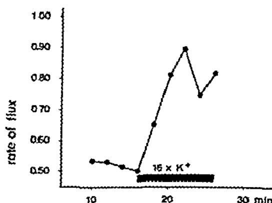
Fig 5

Fig 4 The efflux of ^{36}Cl from the rat portal vein in normal PSS The figure shows an average curve for 6 muscles analysed as described in Fig 3 and computed from the equation ^{36}Cl mmol/kg $W_w = A \times e^{-k_1 t} + B \times e^{-k_2 t} + C \times e^{-k_3 t}$ The values for A B and C and k_1 k_2 and k_3 are given in the text. *Abscissa* *Time in min* *Ordinate* *Tissue radioactivity in mmol/kg* W_w

Fig 5 The efflux of ^{42}K from the rat portal vein in normal PSS The figure shows an averaged curve for 6 muscles analysed as above and computed from the equation ^{42}K (mmol/kg W_w) $= A \times e^{-k_1 t} + B \times e^{-k_2 t}$ For ^{42}K C = 0 *i e* 2 components are sufficient to describe the ^{42}K efflux curve The average values for A B k_1 and k_2 are given in the text Abscissa Time in min Ordinate Tissue radioactivity in mmol/kg W_w

treated in the same way as the sodium efflux curve The mean constants were $k_1 = 2.98 \pm 0.27 \times 10^{[illegible]}$ s^{-1} $k_2 = 6.77 \pm 0.90 \times 10^{-4}$ s^{-1} and $k_3 = 1.055 \pm 0.086 \times 10^{-3}$ s^{-1} The compartments were $A = 55.3 \pm 6.6$ mmol/kg W_w $B = 23.7 \pm 3.8$ mmol/kg W_w and $C = 16.6 \pm 2.6$ mmol/kg W_w Thus for chloride as well as for sodium the sum of A and B is bigger than the amount that can be contained in the extracellular space *In most experiments we observed that the total activity of* ^{36}Cl was considerably *greater than the total chloride content determined* by atomic absorption spectrophotometry the difference roughly corresponding to 200 ml PSS/kg W_w It is likely that more superficial solution is adhering to the tissue when it is carried over from the radioactive solution to the flow chamber than when it is weighed after blotting

Ionic changes and their effect on the ^{36}*Cl efflux* a) [Ca] Five muscles were loaded with ^{36}Cl and the washout studied in Ca free and Ca high (7.5 mM) PSS The efflux curves in these two conditions did not differ significantly from each other nor from the efflux in normal PSS k_1 was 1.07×10^{-2} s^{-1} in Ca free an



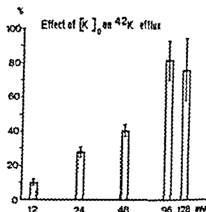


Fig 6

Fig 7

Fig 6 The effect of increased [K]o on the rate of efflux of 42K The [K]o was suddenly in creased to 96 mM after 16 min as shown by the black bar Abscissa Time in min Ordinate Rate of flux in arbitrary units

Fig 7 The relationship between [K]o and the efflux of 42K Abscissa [K]o in mM Ordinate Percentage increase in 42K efflux Vertical bars represent ±S E (n = 6)

10^{-3} s^{-1} in Ca high PSS Ca thus has no effect on the 36Cl efflux and does not seem to be involved in the regulation of chloride permeability

b) [K]o The washout of 36Cl was studied in five muscles which had been in cubated in K high PSS during loading K high solutions are known to increase the permeability to cations in general In this experiment k_3 was 1 80 ± 0 23 × 10^{-3} s^{-1} i e roughly 70 % bigger than in normal PSS Thus in K high PSS the 36Cl efflux is increased reflecting an increase in Cl permeability A lower concentration how ever 24 mM K had no effect on the 36Cl efflux

) *42K efflux in normal PSS* The efflux curve for 12 muscles loaded for 3 h in 42K solution is shown in Fig 5 The potassium efflux curves could always be described by two exponentials thus making easier their interpretation In this experiment k_1 = 1 176 ± 0 083 × 10 s^{-1} and k = 2 15 ± 0 10 × 10^{-4} s^{-1} and A 4 58 ± 0 77 mmol/kg W and B = 47 3 ± 2 4 mmol/kg W_w The total potassium content of these muscles were 52 4 ± 2 5 mmol/kg W_w and the specific activity of potassium in the tissue was close to 100 % Similar results were obtained in other experiments and even if some allowance is made for superficially transferred potassium in the washout experiments it is safe to say that not less than 95 % of the tissue potassium had ex changed during the loading period The first component A was always in these experiments bigger than the amount that could be accounted for by the extracellular space and a rough estimation yields a figure of ≤ 200 ml/kg W_w of radioactive solution carried over

Ionic changes and their effect on the efflux of 42K a) [Ca] Calcium has been proposed as a permeability controlling agent at the smooth muscle membrane

(Brading Bulbring and Tomita 1969) and decreased Ca binding at the membrane is expected to decrease the potassium permeability and therefore to slow down the efflux Six muscles were tested in Ca free solution during the washout of ^{42}K and we found that the rate constant k decreased from $2.15 \pm 0.14 \times 10^{-4}\ s^{-1}$ to $1.70 \pm 0.10 \times 10^{-4}\ s^{-1}$ when Ca was omitted from the solution There was no change in the K content of the tissues in Ca free solution

b) $[K]_o$ There is substantial evidence showing that the potassium permeability is sensitive to changes in the $[K]_o$ (Carmeliet 1960 Casteels 1970) In a series of experiments this was tested by loading the tissue in ^{42}K solution starting the washout in normal PSS and then changing to solutions with increased $[K]_o$ Fig 6 shows the rate of flux for an individual muscle When $[K]_o$ was suddenly changed to 96 mM the rate of efflux doubled and was stable at the new level for as long as $[K]_o$ was increased Similar curves were obtained with 12 24 48 and 128 mM K The results from 5 different experiments are summarized in Fig 7 The increase in the rate of flux was roughly linearly related to the logarithm of the external potassium concentration but levelled off at higher concentrations as indicated by the fact that there was no difference between the effect of 96 and 128 mM K on the ^{42}K efflux

Membrane parameters calculated from efflux data The steady state efflux experiments performed in normal PSS with ^{24}Na ^{42}K and ^{36}Cl can be used to calculate membrane flux permeability and conductance towards a specific ion Furthermore the Goldman equation can be applied and the results compared to known electrophysiological data Certain assumptions have to be made about the ion movements however First of all the late slow linear phase in each efflux curve is taken to represent the transmembrane exchange Secondly the ions are considered as independently moving through the membrane Thirdly the effluxes of potassium and chloride are regarded as passive while that of sodium is probably the result of the sodium pump In a steady state efflux equals influx and the sodium influx has therefore been used to calculate the permeability

The linear phase of the washout when extrapolated to zero tends to overestimate the size of the intracellular fraction according to Huxley (1960 because of interaction between the rapid and the sl w exchange Huxley s correction factor was therefore used in the following form

$$\text{slow component} = \frac{A \times B(k_A - k_B)^2}{A \times k_A^2 + B \times k_B^2}$$

where A is the sum of the first two components in the case of sodium and chloride and the first component in the case of potassium and k_A the corresponding rate constant B is the late linear component and k_B its rate constant

Before the flux can be calculated we need an estimate of the cell surface volume relationship Jonsson (1971 b) reports that the diameter of the smooth muscle cells in the rat portal vein is 4 μ If the cells are considered to be cylindrical

water volume equal to the intracellular volume this would give $V/A = 1\ 06 \times 10^{-4}$ cm On the other hand the dryweight of the tissue represents structural proteins mucopolysaccharides phospholipids and so forth occupying a certain volume from which water is excluded Thus the cell water volume proper is 58 % of the intracellular space and in this case $V/A = 0\ 61 \times 10^{-4}$ cm The flux was calculated according to the formula $M = k \times V/A \times C_i$ (Keynes and Lewis 1951)

The permeability of each ion was calculated from the formula

$$P = \frac{M}{C_i \dfrac{EF/RT}{1 - \exp(-EF/RT)}}$$

The factor $EF/RT/(1-\exp(-EF/RT))$ is a correction factor that applies to a charged particle moving across a membrane separating two areas with different potential At a membrane potential of −45 mV this factor is 0 384 for the efflux of potassium and 2 07 for the efflux of chloride and influx of sodium respectively due to the sign of E which is negative when the fluxes are facilitated by the electric field and positive when they are opposed by it

The calculated permeabilities and intracellular concentrations of sodium potassium and chloride were used to calculate the membrane potential according to the Goldman equation

$$E_m = -\frac{RT}{nF} \ln \frac{P_K [K^+]_i + P_{Na} [Na^+]_i + P_{Cl} [Cl^-]_o}{P_K [K^+]_o + P_{Na} [Na^+]_o + P_{Cl} [Cl^-]_i}$$

It may also be assumed that the current across the membrane in the steady state is zero *i e* that the opposing currents carried by different ions cancel each other In these conditions the conductance of a certain ion *e g* Na may be calculated from the formula

$$G_{Na} = P_{Na} \frac{F}{RT} \frac{F}{E - E_{Na}} \frac{[Na^+]_o - [Na^+]_i \exp(-EF/RT)}{1 - \exp(-EF/RT)}$$

The same formula applies for potassium while for chloride C_i and C_o change places

Application of efflux data From the slow linear exchange of sodium potassium and chloride the intracellular concentrations of these ions were calculated after application of Huxley s correction factor (Table II) The two rapid phases of the washout were considered to be extracellular The rate constant of the slow phase was used to calculate the fluxes and permeability for each ion and in normal solution P_K P_{Na} P_{Cl} was 1 0 034 0 816 The calculated membrane potential was −43 3 mV very close to the measured potential of −45 mV The membrane resistance calculated from the ionic conductances was 68 kΩ cm and although no figures

TABLE II

	Na	K	Cl
Extracellular ion concentration (mM)	137 5	6 0	134 1
Intracellular ion content (mmol/kg W_w)	3 00	45 5	14 7
Cell water concentration (mM)	13 0	197 7	64 0
Transmembrane rate constant ($s^{-1} \times 10^{-3}$)	0 466	0 215	1 055
Apparent fluxes (pmoles cm^{-2} s^{-1})	0 37	2 59	4 12
Corrected fluxes (pmoles cm^{-2} s^{-1})	0 37	2 89	4 12
Permeabilities (cm s^{-1} × 10)	0 130	3 81	3 11
Equilibrium potentials (mV)	+63 0	−93 4	−19 8
Ionic conductances (mho cm $\times 10^{-6}$)	0 32	4 82	9 61
Calculated membrane potential (mV)		−42 3	
Calculated membrane resistance (kΩ cm)		68	

are given in the literature for the portal vein this value comes rather close to the value of 30—50 kΩ cm given by Abe and Tomita (1968) for the taenia coli of the guinea pig If chloride is excluded from the calculations of the resting potential E_m becomes −77 4 mV a value rarely found in electrophysiological experiments on spontaneously active smooth muscles in normal PSS On the other hand exclusion of sodium from the calculations yields a value of $E_m = -44\ 4$ mV Thus the distribution and permeabilities of potassium and chloride seem sufficient to predict the resting potential

Discussion

The results given in this paper show that the intracellular concentrations of sodium potassium and chloride calculated from the total ion content and the extracellular space are more or less similar to values found in the taenia coli of the guinea pig (Casteels and Kuriyama 1966 Goodford 1964 Buck and Goodford 1966 Brading 1971) and in cat and guinea pig myometrium (Bulbring Casteels and Kuriyama 1968)

When intracellular concentrations were used to calculate the equilibrium potentials it was found that no single ion could be used to predict the membrane potential In nerve (Hodgkin 1951) the potential can be predicted from the equilibrium potential for potassium across the membrane with a slight discrepancy due to sodium permeability in the resting membrane In smooth muscle however a more complex situation exists and an analysis of sodium potassium and chloride movements under similar conditions becomes necessary

In the present experiments intracellular sodium was estimated from the slow fraction of the efflux curve instead of from the ionic content and extracellular space and the former value used for calculations of the sodium equilibrium potential and sodium transmembrane flux From the data presented here it looks as if sodium plays a very small part in determining the level of the resting potential Errors in the estimation of intracellular sodium content will then have an

cant effect on the calculations of E_m

The interpretation of the first two components of the sodium efflux is highly conjectural Brading and Jones (1968) suggested that the first two phases of sodium efflux in taenia coli are due to bulk diffusion of tracer from the tissue, and in a recent paper Brading (1971) has applied this treatment to potassium and chloride fluxes as well For reasons given above no definite meaning has been given to the first two phases except that they are extracellular In the present experiments the slow exchange of sodium had a half time of 40 min while Jonsson (1971a) reports that the half time for the slow sodium exchange is 14 8 min in the portal vein at 15° C In taenia coli of the guinea pig Brading (1971) has found a half time of 19 5 min while Casteels (1970) gives the altogether different figure of 2 9 min These discrepancies may reflect differences in experimental procedure In the work by Brading and in the present work a constant flow chamber was used in which the muscle was firmly fixed throughout the experiment In most efflux procedures however the tissue is moved from one test tube to the next and it is possible that the frequent moving stimulates the tissue to such an extent that ion effluxes especially sodium efflux are affected

The most interesting feature in the results described above is the obvious role played by chloride The total chloride content is high and the difference between flux data and ion content determinations not too large Goodford (1964) reported that the chloride content of guinea pig taenia coli was 103 mmol/kg W_w in normal PSS Casteels (1970) reported 72 4 mmol/kg W_w for guinea pig taenia coli and 71 0 mmol/kg W_w for guinea pig was deferens The chloride content in cat uterus was 86 6 mmol/kg W_w in the virgin cat and 96 mmol/kg W_w in early pregnancy (Bulbring *et al* 1968) In the rat portal vein the total chloride content was 96 4 mmol/kg W from ion content measurements but usually larger from tracer experiments The third and slowest component of the ^{36}Cl efflux was similar in size from one experiment to the next with a small variation in half time between experiments but the size of the first two compartments varied from one experiment to another, usually between 80 and 100 mmol/kg W_w The reason for this is to be found in a varying amount of superficial solution carried over from the radioactive solution In the present work therefore only the slow phase was considered to be transmembranal and used for calculations of ion permeability and equilibrium potential Further justification for this approach is given by the fact that a good prediction of the membrane potential is obtained

The high intracellular concentration of chloride raises the question of an active transport mechanism for this ion directed into the cell Such a transport mechanism has been proposed by Casteels (1965) He found that DNP Na free solution K free solution and low temperature all decreased ^{36}Cl uptake in taenia coli of the guinea pig Ouabain had a similar effect More recent work indicates that the uptake of chloride may be coupled to the potassium uptake Thus the efflux of potassium is slowed down if chloride is substituted by a less permeant anion like ethane sulphonate and slightly increased if a more permeant anion like NO_3 is used

instead of chloride (Casteels 1970) Those findings however have to be reconciled with the fact that permeant anions like nitrate increase the membrane potential contrary to expectations (Kuriyama 1963) There is a possibility that permeant anions can be used as counter ions for positive charges in the membrane pore making the passage of K ions easier At present the evidence for such a model is only suggestive

In the rat portal vein ouabain was without effect on the chloride content This need not mean that there is no active transport mechanism for chloride and other metabolic inhibitors have to be tested in their effects on ion content as well as on chloride efflux and uptake This will be subject to further investigations

The potassium distribution in the rat vein can be explained by a two-compartmental model and the values for the intracellular slow fraction agree with earlier data from the portal vein (Jonsson 1971a) and with data from other tissue as well (Brading 1971 Casteels 1970) The potassium permeability decreases when $[Ca^{++}]_o$ is reduced and the fact that there was no such dependency for chloride is against the idea of a coupled transport of portassium and chloride

The fact that chloride in the rat portal vein is present in a high intracellular concentration together with the rapid exchange of chloride across the membrane makes this ion interesting from the point of view of drug actions and the effects of other environmental changes The chloride conductance is also 65 % of the total calculated membrane conductance Ohashi (1970) concluded that 40 % of the conductance in the taenia coli was due to chloride the rest being due to potassium Since chloride is even more important in determining the level of the resting potential in the portal vein than in taenia coli (Brading 1971) the question arises whether changes in chloride permeability or distribution are involved in the action of transmitters on the vascular smooth muscle membrane This will be further discussed in a following paper (Wahlstrom 1973)

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References

ABE Y and T TOMITA Cable prop[illegible] J Ph[illegible] Lond [illegible] 87—100

BRADING A F A constant flow a[illegible] J Physiol Lond [illegible]

BRADING A F Analysis of the eff[illegible] muscle in normal and hypert[illegible] solution J Physiol Lond [illegible] 393—41[illegible]

BRADING A F and A JONES Distribution and kinetics of CoEDTA [illegible] and its use as an extracellular mark[illegible] J Physiol Lond [illegible] 200 [illegible]

BRADING A F E B [illegible] and T TOMITA The effect of sodium [illegible] potential of the smooth muscle of the guinea pig taenia coli J Physiol Lond [illegible] 637—65[illegible]

BUCK B and P J GOODFORD The distribution of ion[illegible] taenia coli J Physiol (Lond) 196[illegible] 183 551—56[illegible]

BULBRING E R CASTEELS and H KURIYAMA Membrane potential and ion content in cat and guinea pig myometrium and the response to adrenaline and noradrenaline *Brit J Pharmacol* 1968 *34* 388—407

CARMELIET E L influence de la concentration extracellulaire du K sur la permeabilite de la membrane des fibres de Purkinje de mouton pour les ions 42K *Helv physiol pharmacol Acta* 1960 *18* C15

CASTEELS R The chloride distribution in the smooth muscle of the guinea pig s taenia coli *J Physiol* (Lond) 1965 *178* 10—11P

CASTEELS R The action of ouabain on the smooth muscle cells of the guinea pig s taenia coli *J Physiol* (Lond) 1966 *184* 131—142

CASTEELS R Calculation of the membrane potential in smooth muscle cells of the guinea pig s taenia coli by the Goldman equation *J Physiol* (Lond) 1969 *205* 193—208

CASTEELS R The relation between the membrane potental and the ion distribution in smooth muscle cells In *Smooth Muscle* Arnold Ltd London 1970 70—99

CASTEELS R. and H KURIYAMA, Membrane potential and ion content in the smooth muscle of the guinea pig's taenia coli at different external potassium concentrations *J Physiol* (Lond) 1966 *184* 120—130

GOLDMAN D E Potential impedance and rectification in membrane *J gen Physiol* 1943 27 37—60

GOODFORD P J Chloride content and 36C uptake in the smooth muscle of the guinea pig taenia coli *J Physiol* (Lond) 1964 *170* 227—237

HALJAMAE H B JOHANSSON O JONSSON and H HOCKERT The distribution of sodium potassium and chloride in the smooth muscle of the rat portal vein *Acta physiol scand* 1970 *78* 255—268

HODGKIN A L. The ionic basis of electrical activity in nerve and muscle *Biol Rev* 1951 26 339—409

HODGKIN A L and P HOROWICZ The influence of potassium and chloride ions on the membrane potential of single muscle fibres *J Physiol* (Lond) 1959 *148* 127—160

HUXLEY A F In *Mineral Metabolism* 1960 *1* 163—167

JONSSON O Effects of variations in the extracellular osmolality on the ionic permeability of vascular smooth muscle *Acta physiol scand* 1971 a *81* 405—421

JONSSON O Effects of variations in the extracellular osmolality on the permeability to non electrolytes of vascular smooth muscle *Acta physiol scand* 1971 b *81* 528—540

KEYNES R D and P R LEWIS The resting exchange of radioactive potassium in crab nerve *J Physiol* (Lond) 1951 *113* 73—98

KURIYAMA H The influence of potassium sodium and chloride on the membrane potential of the smooth muscle of *taenia* coli *J Physiol* (Lond) 1963 *166* 15—28

OHASHI H An estimate of the proportion of the resting membrane conductance of the smooth muscle of guinea pig taenia coli attributable to chloride *J Physiol* (Lond) 1970 *210* 405—419

WAHLSTROM B A The effects of changes in the ionic environment on venous smooth muscle distribution of sodium and potassium *Acta physiol scand* 1971 *82* 382—392

WAHLSTROM B A The effects of noradrenaline on ion content and 42K and 36Cl effluxes in the rat portal vein *J Physiol* (Lond) 1972 *226* 63—64P

WAHLSTROM B A A study on the action of noradrenaline on ionic content and sodium potassium and chloride effluxes in the rat portal vein *Acta physiol scand* 1973 *89* In press

Acta physiol scand 1973 89 449—456

From the Department of Physiology University of Goteborg Sweden

Vascular Reactions in the Small Intestine during Vasodilatation

By

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Abstract

Biber B J Fara and O Lundgren *Vascular reactions in the small intestine during vasodilatation* Acta physiol scand 1973 *89* 449—456

The reactions within the consecutive vascular sections of the mall intestine of the cat were studied during vasodilatations induced by secretin and cholecystokinin infused i a or releas d endogenously or by transmural electrical field and mucosal mechanical stimulations All these potentially physiological stimuli evoked a response pattern similar to that of i a. infused 5 hyd oxytryptamine and characterized by a relaxation of the resistance vessels as well as of the precapillary sphincters as reflected in an increased capillary filtration coefficient A small increase of regional blood volume was also observed during most vasodilatations On the basis of these experiments it is impossible to exclude any of the abovementioned mechanisms in the discussion of the cause of the functional hyperemia of the gut It is proposed that this vasodilatation depends partly upon a release of intestinal hormones partly upon local mechanisms evoked by mechanical and poss ble also by chemical stimulation of the intestinal mucosa

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It is well known that intestinal blood flow increases during digestion and it is reasonable to assume that this functional hyperemia also involves regional blood flow adjustments of considerable importance also for intestinal function It has further been reported that regional blood flow increases can be induced by several different types of intestinal stimuli such as 1) cholecystokinin (CCK) and secretin (Fara Sonnenschein and Rubinstein 1972) released from the intestine by intra luminal fat and amino acids 2) mechanical stimulation of the mucosa (Biber Lund gren and Svanvik 1971) and 3) transmural electrical stimulation (Biber Fara and Lundgren 1973 a) In addition intestinal blood flow increases of a similar magni tude are induced by i a administration of 5 hydroxytryptamine (5 HT Biber Fara and Lundgren 1973 b) also shown to be present in considerable amounts in the intestinal wall (Erspamer 1966 Gershon and Ro s 1966 Vialli 1966 Penttila and Lempinen 1968)

It is therefore apparent at different types of local stimuli may contribute to the physiological control of intestinal blood flow Whether the mentioned vasodilator influences create largely the same pattern of vascular changes and whether a com

mon vasodilator mechanism is then involved is not known In order to elucidate this question a comparison was made of the effects induced by CCK secretin 5 HT field stimulation and mechanical mucosal stimulation concerning the consecutive vascular sections of the cat small intestine

Methods

The experiments were performed on 25 cats of both sexes deprived of food for at least 24 h prior to the experiment Anesthesia was induced by ether and continued by chloralose i v (50—60 mg/kg b w) The operative procedures were similar to those previously described in detail (Biber *et al* 1971) An intestinal segment close to the flexura jejuno-duodenalis and weighing 15—30 g with its lymph nodes was chosen for the experiment and left *in situ* while the rest of the intestine the spleen and the greater omentum were extirpated

Recordings of vascular parameters Jejunal venous outflow was measured by an optical drop recorder operating an ordinate writer and arterial blood pressure monitored from the left femoral artery by means of a Statham high pressure transducer (P23AC) both recordings being made on a Grass polygraph

To measure intestinal volume the jejunal segment alone or together with its lymph nodes was placed in a lucite plethysmograph filled with Tyrode s solution at 38 C volume changes being recorded on the Grass polygraph The venous pressure could be intermittently raised for CFC measurements (for details see Folkow Lundgren and Wallentin 1963 or Haglund and Lundgren 1972) Injections or infusions to the jejunal segment could be made into the superior mesenteric artery through a cannula in a branching vessel

The splanchnic nerves were cut while the vagal intestinal innervation was left intact. In some experiments the left adrenal gland was denervated and the right one excluded from the circulation by ligatures

Transmural field stimulation Most experiments were arranged to allow transmural electrical field stimulation inside the plethysmograph A soft plastic tube (outer diameter 2 mm) with a flattened silver wire wrapped around its full length was placed inside the intestinal lumen usually serving as the cathode. A flexible silver wire closely enwrapping the jejunal preparation from the outside served as the outer anodal electrode Electrical stimulation was usually applied at a strength of 60—100 mA using a frequency of 60—80 Hz with a pulse duration of 8—10 ms these parameters being chosen to elicit maximal vasodilator response (for details see Biber *et al* 1973 a)

Intraluminal perfusion experiments In 6 cats a 45—60 cm duodenal jejunal segment was perfused intraluminally with either isotonic 0 15 N HCl (9 perfusions) or with isotonic 0 2 M L-phenylalanine (7 perfusions) at 0 5—1 ml/min while an immediately distal intestinal segment was used for recording changes in intestinal blood flow blood volume and changes in perfused capillary exchange surface For this purpose a cannula was introduced into the duodenum via the stomach with subsequent ligation of the pyloric junction while another cannula was placed in the distal end of the segment to be perfused The segment was first flushed with saline as a control and also between changes of the perfusion fluid Additionally in most cats the pancreatic duct was cannulated to record pancreatic secretion and after ligation of the cystic duct the gallbladder was cannulated to record its intraluminal pressure (motility)

Mechanical stimulation of the intestinal mucosa In 4 expts intermittent mechanical stimulations of the intestinal mucosa were performed while the intestinal segment was placed inside the plethysmograph A 3 cm long plastic tube with an outer diameter of 5 mm was cautiously pulled back and forth along the intestinal lumen by means of soft strings tied to its ends (for details see Biber *et al* 1971)

Drugs Atropine atropine sulphate 0 5—1 mg/kg b w) was given routinely to reduce intestinal motility which would otherwise sometimes interfere with the volume recordings in the plethysmograph The intestinal hormones cholecystokinin pancreozymin (CCK) and secretin were obtained from the Gastro intestinal Research Unit Karolinska Institutet Stockholm 5-hydroxytryptamine creatinine sulphate (May & Baker) was given as close i a injections or infusions corresponding to 10—50 μg/min

Results

A Vascular effects of field stimulation and 5 HT In 10 cats electrical field stimulation across the jejunal segment evoked a 60—150 per cent increase in blood flow

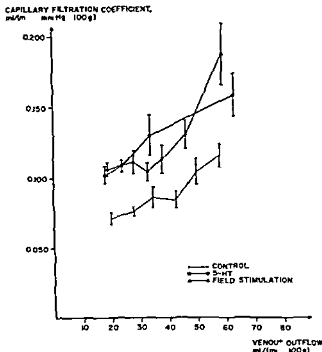


Fig 1 Cumulated data on the correlation between total intestinal blood flow and capillary filtration coefficient during control conditions (bottom curve) during graded i a infusions of 5-hydroxytryptamine (5 HT) and during transmural electrical field stimulation. Bars indicate ± S E Each point represent 10—18 observations

which was fully developed within 10 s and in other respects showed characteristics similar to those earlier described (Biber *et al* 1973 a) During the vasodilatation the capillary filtration coefficient (CFC) of the segment always increased above control for any given blood flow level (see Fig 1) Upon stimulation regional tissue volume increased rapidly in all probability reflecting an augmented regional blood content of the order of about 1 ml/100 g tissue or a 10—15 per cent increase of the blood content (*cf* Folkow *et al* 1963) During a prolonged field stimulation a slow continuous increase of tissue volume was usually observed amounting to 0 8—1 2 ml/min × 100 g when the observed blood flow increase was 10 ml/min × 100 g or more However this volume change was most likely due to a stimulation artifact since a repetition of the same field stimulation during a period of complete circulatory obstruction evoked a similar volume increase This possibly reflected the formation of gas at the cathode

Close i a. infusions of 5 HT at 20—50 μg/min produced intestinal vasodilatations consistently accompanied by an increased CFC which closely mimicked the effects induced by field stimulation (Fig 1) Lower concentrations of 5 HT (10—15 μg/min) often increased CFC but usually not total intestinal blood flow as also reported earlier (Biber *et al* 1973 b)

The capacitance vascular response to 5 HT infusion varied Sometimes signs of a slight venoconstriction appeared while on other occasions the reverse was true then amounting to only some 0 2 ml/100 g In addition a more continuous phase of intestinal volume increase was usually seen during the influence of 5 HT of the

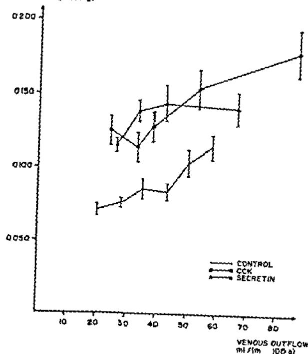


Fig 2 Cumulated data on the correlation between total intestinal blood flow and capillary filtration coefficient during control conditions (bottom curve) and during graded i a infusions of cholecystokinin (CCK) or secretin Bars indicate ± S E Each point represents 10—18 observations

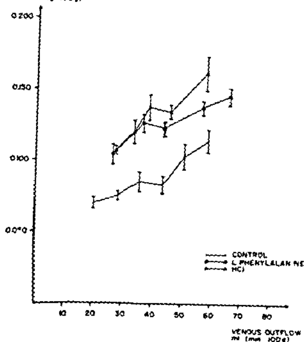


Fig 3 Cumulated data on the correlation between total intestinal blood flow and capillary filtration coefficient during control conditions (bottom curve) and during intraluminal perfusion of L-phenylalanine or HCl The luminal perfusions of the gut cause an endogenous release of secretin and cholecystokinin Bars indicate ± S E Each point represents 10—18 observations

of 0.4—0.8 ml/min×100 g when intestinal blood flow was increased some 20—30 %. In some experiments a tissue volume increase was registered even in the absence of a blood flow effect of 5 HT.

B. Vascular effects of mechanical stimulation. Pulling a closely fitting PVC tube along the lumen of the jejunal segment when placed inside the plethysmograph evoked a 30—140 % increase in intestinal blood flow as also reported earlier (Biber *et al.* 1971). The flow increase was always accompanied by a CFC increase in a relationship that closely mimicked that induced by electrical field stimulations, 5 HT, secretin and CCK (Fig. 1, 2 and 3). It was not possible to estimate the blood volume changes of mechanical stimulation since the stimulation procedure induced large alterations of the tissue volume.

C. Vascular effects of intestinal hormones. Close i.a. infusions (3—12 U/kg×h) of CCK and secretin increased intestinal blood flow and CFC such that the CFC values were always higher than control for any given blood flow level (Fig. 2). The CFC increase was sometimes apparent before the blood flow increase and at hormone administrations below 3 U/kg×h a CFC increase was usually appearing without any significant blood flow increase. Within some minutes of CCK infusion the gallbladder pressure began to increase while there was no substantial pancreatic secretion. Secretin, on the other hand, increased the pancreatic secretion substantially but did not affect gallbladder motility. After stopping the hormone infusions blood flow returned to control within 3—5 min, whereas the return of the CFC values was delayed another 4—6 min.

A 15 min intraluminal perfusion of a duodenal jejunal segment with L-phenylalanine releasing CCK from the intestinal wall (Meyer and Grossman 1970) produced an increase of gallbladder pressure within 5—7 min after the start of the perfusion but no perceptible pancreatic secretion occurred. Concomitantly CFC in the distal intestinal segment, which was excluded from the luminal perfusion (see Methods), was augmented. After 10 min of luminal perfusion blood flow of the segment used for the recordings of vascular effects had also increased while arterial pressure remained unchanged. At the end of the luminal perfusion of the proximal segment blood flow returned to control in the distal segment 5—10 min before CFC was normalized.

Intraluminal perfusion of the proximal segment with HCl releasing CCK and secretin (Wang and Grossman 1951, Berry and Flower 1971) evoked a similar response pattern with the addition of a substantial increase in pancreatic secretion beginning within 5 min after the start of perfusion and continuing for 5—10 min after the HCl perfusion had ended. Here also CFC in the distal segment increased before its blood flow increased. Control saline perfusions of the proximal segment before each procedure produced none of these responses. Fig. 3 summarizes the correlation between intestinal blood flow and CFC in these experiments. It is apparent that the observed CFC values for a given blood flow level were as large as those seen during i.a. infusion of CCK and secretin (Fig. 2).

In many instances it was possible to estimate the increase of regional blood volume caused by the CCK or secretin appearing as a rapid augmentation of tissue volume concomitant to the blood flow increase For both hormones the volume increase amounted to 0 7—0 8 ml/100 g (about 10 % increase of regional blood volume) at most while blood flow increased from about 25 to approximately 40 ml/min × 100 g Due to the slow onset of the vascular effects during intraluminal infusions of HCl and L phenylalanine no clearcut changes of regional blood content could be deduced from the changes in tissue volume in these experiments

In most experiments a slow continuous increase of tissue volume was observed during the intravascular infusions of the hormones usually amounting to 0 3—0 6 ml/min × 100 g and always accompanied by a blood flow increase

Discussion

Using a combination of direct blood flow measurement and a plethysmographic recording technique the present study enabled continuous and simultaneous determinations of reactions within intestinal resistance exchange and capacitance blood vessels while the intestine was exposed to various types of stimuli The changes in the intestinal resistance vessels were measured by recording perfusion pressure and blood flow while alterations of the capacitance vessels (*i e* of blood content) were reflected in terms of the rapid changes in tissue volume occurring along with the changes in blood flow resistance The capillary filtration coefficient (CFC) measuring the hydralic conductivity of the exchange vessels reflects in most cases the tone of the precapillary sphincter sections (Mellander 1960 Folkow 1967)

The vascular response pattern evoked by all the dilatory stimuli used was more or less the same throughout this study Fig 1 2 and 3 clearly demonstrate that blood flow and CFC changes induced by CCK and secretin whether exogenously given or endogenously released by the intraluminal perfusions of HCl or L phenylalanine were of the same order of magnitude as those evoked by electrical field stimulations Similar vascular reactions were also observed after mechanical mucosal stimulations A moderate dilatation of the regional capacitance vessels (veins) was also registered during field stimulation and during intravascular infusion of the hormones *Thus similar hemodynamical adjustments were observed in response to* the different potential physiological dilatory mechanisms of the gut making it impossible to exclude any of them when discussing the cause of vasodilatation occurring during normal digestion This might imply that the functional hyperemia of the small intestine is dependent partly upon a release of hormones increasing blood flow diffusely throughout the gut and partly upon local mechanisms evoked by mechanical and perhaps also local chemical influences of the contents acting directly on the intestinal mucosa

Furthermore the intestinal vascular responses to CCK secretin to local mechanical and electrical field stimulations are not only similar from a hemodynamic point of view but have also pharmacological properties in common This type of vasodilator response has been shown to be independent of cholinergic and adrenergic

receptor mechanisms and all seems to be abolished by 5 HT blocking agents (Biber et al 1971, 1972 Fara et al 1972) These two findings point to a common mediator mechanism the nature of which is still unknown However, 5 HT when administered intraarterially to the small intestine causes a closely corresponding vascular response pattern as demonstrated earlier (Biber et al 1973 b) and also in the present study

The magnitude of CFC is determined not only by the number and size of pores per capillary surface area but also by the surface area perfused which again is a consequence of the tone of the precapillary sphincters sections of the microvessels As the precapillary sphincter sections *per se* seem to contribute relatively little to *the total intestinal resistance to blood flow net changes in resistance or blood flow* do not necessarily correspond to any CFC changes (*cf* Folkow *et al* 1964) Thus during infusions of CCK secretin and 5 HT a CFC increase was sometimes observed at dosages lower then those needed to produce changes in intestinal blood flow, perhaps suggesting a greater sensitivity of the precapillary sphincter sections than of the resistance vessels to these agents However the mentioned difference might also reflect an increased capillary permeability induced by low concentrations of these substances

A slow continuous increase of tissue volume was recorded in many experiments during i a infusions of 5 HT secretin and CCK as well as during field stimulation In the latter case the volume change was at least partly due to a stimulation artifact probably caused by a gas formation at the cathode while the significance of the other observations is not yet established Three possible explanations exist

Firstly, tissue volume may increase as a result of an intestinal secretory response However secretin and CCK given in doses up to 4 U/kg×h causes a maximal ecretory response of the Brunner s glands in the cat duodenum of about 0 5—0 8 ml/h (Stenning and Grossman 1969) *i e* only a fraction of the tissue volume augmentation seen in this study even when correction is made for differences in tissue weight Since the duodenal mucosa represents the most secretory portion of the intestinal wall it seems very unlikely that the jejunal mucosa could have a secretory response that is more pronounced than that seen in the duodenum Secondly the pre to postcapillary resistance ratio may decrease as a consequence of an increased motility increasing mean capillary hydrostatic pressure and hence the rate of trans capillary fluid filtration However of the va odilatory mechanisms studied here only 5 HT may induce motility in atropinized cats The pre to postcapillary resistance *ratio may also decrease as a con equence of the vasodilatation if this is mainly* localized to the precapillary portion of the intestinal vasculature Thirdly an increased size of the capillary pores may decrease effective plasma colloid pressure leading to an increased filtration of fluid Such a mechanism was sugge ed by the observation that CFC and/or tissue volume in some instances increased in the absence of an effect on blood flow when infusing 5 HT secretin or CCK From this circumstantial discussion one may tentatively conclude that the observed continuous tissue volume increa es are partly due to a precapillary vasodilatation causing

an increase of mean capillary pressure and partly due to an increased capillary porosity

Intraduodenal instillation of L phenylalanine is known to selectively release CCK in the dog (Meyer and Grossman 1970) and cat (Fara *et al* 1972) and HCl has been demonstrated to release both secretin and CCK (Wang and Grossman 1951 Berry and Flower 1971) The current experiments in which a proximal duodenal jejunal preparation was perfused with L phenylalanine or HCl substantiate the mentioned release of CCK and/or secretin but also demonstrate that this endogenous hormone release induces the same effects on the intestinal vasculature as when the hormones are administered exogenously

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References

BERRY H and R J FLOWER The assay of endogenous cholecystokinin and factors influencing its release in the dog and cat *Gastroenterology* 1971 *60* 409—420

BIBER B J FARA and O LUNDGREN 5 Hydroxytryptamine and intestinal blood flow *Acta physiol scand* 1972 *84* 9A—10A

BIBER B J FARA and O LUNDGREN Intestinal vasodilatation in response to transmural electrical field stimulation *Acta physiol scand* 1973 a *87* 277—282

BIBER B J FARA and O LUNDGREN Intestinal vascular responses to 5 Hydroxytryptamine *Acta physiol scand* 1973 b *87* 526—534

BIBER B O LUNDGREN and J SVANVIK Studies on the intestinal vasodilatation observed after mechanical stimulation of the mucosa of the gut *Acta physiol scand* 1971 *82* 177—190

ERSPAMER V Occurrence of indolealkylamines in nature In *Handbook of Experimental Pharmacology* 1966 XIX 132—181

FARA J W R R SONNENSCHEIN and E H RUBINSTEIN Intestinal hormones in mesenteric vasodilation after intraduodenal agents *Amer J Physiol* 1972 *223* 1058—1067

FOLKOW B Regional adjustments of intestinal blood flow *Gastroenterology* 1967 *52* 423—432

FOLKOW B O LUNDGREN and I WALLENTIN Studies on the relationship between flow resistance capillary filtration coefficient and regional blood volume in the intestine of the cat *Acta physiol scand* 1963 *57* 270—283

FOLKOW B D H LEWIS O LUNDGREN and I WALLENTIN The effect of the sympathetic vasoconstrictor fibres on the distribution of capillary blood flow in the intestine *Acta physiol scand* 1964 *61* 458—466

GERSHON M D and L L ROSS Location of sites of 5-hydroxytryptamine storage and metabolism by radioautography *J Physiol* (Lond) 1966 *186* 477—430

HAGLUND L and O LUNDGREN Reactions within consecutive vascular section of the small intestine of the cat during prolonged hypotension *Acta physiol scand* 1972 *84* 151—163

MELLANDER S Comparative studies on the adrenergic neurohormonal control of resistance and capacitance vessels in the cat *Acta physiol scand* 1960 *50* Suppl 176

MEYER J H and M I GROSSMAN Comparison of D and L phenylalanine as pancreatic stimulants *Gastroenterology* 1970 *58* 1046

PENTTILA A and M LEMPINEN Entero-chromaffin cells and 5 hydroxytryptamine in the human intestinal tract *Gastroenterology* 1968 *54* 375—381

STENING G F and M I GROSSMAN Hormonal control of Brunner's glands *Gastroenterology* 1969 *56* 1047—1052

WANG C C and M I GROSSMAN Physiological determination of the release of secretin and pancreozymin from intestine of dogs with transplanted pancreas *Amer J Physiol* 1951 *164* 527—545

VIALLI M Histology of the enterochromaffine cell system In *Handbook of Experimental Pharmacology* 1966 XIX 1 45

Acta physiol scand 1973 89 457—481
From the Department of Physiology University of Goteborg S eden

Recurrent Control from Motor Axon Collaterals of Ia Inhibitory Pathways to Ventral Spinocerebellar Tract Neurones

By

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Abstract

Gustafsson B and S Lindstrom *Recurrent control from motor axon collaterals of Ia inhibitory pathways to ventral spinocerebellar tract neurones* Acta physiol scand 1973 *89* 457—481

The effects of impulses in recurrent motor axon collaterals on transmission in different inhibitory pathways to ventral spinocerebellar tract (VSCT) neurones were investigated in the cat by conditioning of IPSPs evoked in intracellularly recorded VSCT cells D synaptic IPSP from large muscle spindle (Ia) afferents were depressed in many but not all VSCT cells following an antidromic stimulation of ventral roots The effect was found in VSCT neurones which themselves did not receive recurrent inhibition from motor axon collaterals In cells with affected Ia IPSPs also some polysynaptic IPSPs evoked from ipsi and contralateral group II muscle afferents and low th eshold cutaneous afferents were depressed by a ventral root volley as well as disynaptic IPSPs from fibres descending on the ipsilateral side of the spinal cord In unanesthetized preparations recurrent facilitatory potentials similar to those in motoneurones were evoked in VSCT neurones with Ia IPSPs The findings indicate that some VSCT neurones e eive collate al connexions from the interneurones h ch mediate Ia recip ocal inhibition to motoneurones and support the hypothesis that the VSCT conveys information about transmis sion in inhibitory reflex pathways to motoneurones (Lundberg 1971)

Lundberg (1971) has recently presented a hypothe is postulating that the ventral spinocerebellar tract (VSCT) signals segmental interneuronal activity in the spinal cord More specifically it was suggested that the VSCT conveys information about the effectiveness of the transmission in inhibitory interneuronal pathways to motoneurones and possibly also about excitatory and inhibitory effects converging onto motoneurones This type of information might be valuable for cerebellum since most of the descending control of motoneurones seems to be mediated through interneurones interpo ed in different segmental reflex pathways (Lundberg and Voorhoeve 1962 Lundberg 1966 1969 Hongo *et al* 1972 Hultborn 1972 b) The possibility that ascending pathways may relay information about interneuronal transmission to motoneurones has been discussed earlier in relation to effects f

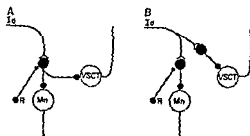


Fig 1 Schematic representation of two possible alternatives for the organization of the Ia inhibitory pathways to motoneurones (Mn) and VSCT neurones A inhibition to both types of cells mediated through common interneurones which receive recurrent inhibition through Renshaw cells (R) B inhibition mediated through separate interneurones those terminating on VSCT cells lacking inhibition from Renshaw cells Further explanation in text

flexor reflex afferents (FRA) on ascending pathways (Lundberg 1959 1964 Oscarsson 1967 1968 Miller and Oscarsson 1970) among them the VSCT which receives inhibition from the FRA (Oscarsson 1957 1960, Magni and Oscarsson 1961 Eccles *et al* 1961, Lundberg and Weight 1971) Unfortunately the organization of the FRA pathways to motoneurones is still too little known to allow critical testing of this important idea The new VSCT hypothesis has the advantage that it includes one of the simplest and best known interneuronal reflex pathways in the spinal cord the disynaptic reciprocal inhibitory pathway from large muscle spindle (Ia) afferents to motoneurones In fact this pathway has in many ways served as a model for the new VSCT hypothesis

Some VSCT neurones receive monosynaptic excitation and/or disynaptic inhibition from large muscle spindle (Ia) afferents (Lundberg and Weight 1971) One subgroup of these cells has the unique convergence of Ia excitation and inhibition from afferents in the same nerve Lundberg (1971) suggested that the Ia excitation of VSCT cells is related to the excitation of Ia inhibitory interneurones from these afferents and that the corresponding inhibition of the VSCT cells is evoked through collateral connexions from the Ia inhibitory interneurones VSCT cells with Ia excitation and inhibition from the same nerve would thus through collateral connexions receive both the excitatory input and the inhibitory output from the interneurones in the Ia inhibitory pathway to motoneurones Such cells could be considered as input output comparators for this pathway Other Ia excited VSCT neurones may receive only the synaptic effects converging onto the Ia inhibitory interneurones or a fraction of these effects These cells may in a wider sense contribute to the comparator function of the VSCT by providing cerebellum with a reference for the input output comparing cells

The main aim of the present study was to test the important assumption in Lundberg's hypothesis that the Ia inhibition of VSCT neurones is evoked through collateral connexions from the interneurones which relay reciprocal Ia inhibition to motoneurones Our indirect approach has been to compare the pattern of synaptic convergence on the interneurones mediating Ia inhibition to VSCT neurones and to motoneurones setting out from the finding that the Ia inhibitory interneurones to the latter cells are effectively inhibited from recurrent motor axon collaterals through Renshaw cells (Hultborn *et al* 1971 a b Hultborn 1972 a) If the Ia inhibition of

the VSCT cells is relayed through the same interneurones (Fig 1 A) then the Ia IPSPs in VSCT neurones should be depressed by conditioning antidromic impulses in ventral root (cf Hultborn et al 1971 a) If no such effect occurs then the Ia inhibition of the VSCT cells must be mediated by interneurones other than those relaying Ia inhibition to motoneurones (Fig 1 B) and consequently one of the major assumptions in the new VSCT hypothesis is false The results will demonstrate that Ia IPSPs in many VSCT neurones are susceptible to recurrent depression as required by the hypothesis This convergence however is not found in all Ia inhibitory pathways to the VSCT in a forthcoming paper it will be shown that a specific group of Ib excited VSCT neurones receive Ia inhibition in accordance with the alternative in Fig 1 B (Lindström and Schomburg 1973 b) A short preliminary note of the present findings has been published (Gustafsson and Lindström 1970)

Methods

Preparation The material was collected in experiments on [illegible] adult cats some primarily used for other purpose The animals were operated under ether anaesthesia which was discontinued about 2—3 hours before the recording started 11 cats were subsequently anaesthetized with alpha-chloralose (i v initially 50—60 mg/kg and additional 10—[illegible]0 mg/kg after [illegible] hrs while 6 cats received pentobarbitone sodium (Nembutal Abbott [illegible] initially 30 mg/kg with supplementary doses as needed) Five cats were anemically decerebrated as described by Andén et al (1966) and maintained unanesthetized during the first part of the recording session Later small doses of Nembutal were supplied i v In most experiments [illegible] the spinal cord was transected at the level of Th12—Th13 In the remaining 5 cats the right spinal half contralateral to the recording side was left intact to allow antidromic activation of VSCT cells from cerebellum (dorsal funiculi and left spinal half transected at Th[illegible]—Th13) Except for the occurrence of recurrent facilitatory potentials (RFPs) in some cells in the unanesthetized animals (see section III) there was no difference between the results in the different preparations All cats were immobilized with gallamine triethiodide (Flaxedil May & Baker Ltd) and artificially respired The maintenance of the preparation was otherwise as described previously (Hultborn et al 1971 a)

The spinal cord was exposed by laminectomy of the Th1[illegible] — Th13 and of the L5 S1 vertebrae Caudal to the transection at Th12—Th13 the dorsal funiculi were removed for about [illegible] and the two spinal halves were mounted on separate bipolar stimulating electrodes In the [illegible] experiments where the right spinal half was left intact the corresponding stimulating electrodes were placed on the surface of the cord In these experiments the surface of the left anterior lobe of cerebellum was exposed by craniotomy posterior occipital [illegible] and partial removal of the bony tentorium The ventral roots L5 S1 on the left side were transected and their proximal ends mounted on stimulating electrodes Usually the L7 S1 ventral roots were mounted on a common pair of electrodes Several *muscle and skin nerves in the left hindlimb* (as listed in the abbreviations) were dissected and mounted on electrodes for electrical stimulation Buried electrodes were used for the ventral nerves The skin flaps around the exposed tissues in the back and the hindlimb were sewed up to form pools which were filled with warm liquid paraffin

Stimulation and recording The peripheral nerves ventral roots and spinal halves were stimulated with square pulses of 0 1 ms duration The strength of the nerve stimuli is indicated in multiples of threshold strength Incoming and descending volleys were recorded from the surface of the spinal cord between a ball tipped silver electrode [illegible] to the entry of the L6 or L7 dorsal roots and an indifferent electrode in the [illegible] muscles Differentiation between afferent fibre groups was done as described by Bradley [illegible] (1953) Eccles et al (1957 a, c) and R M Eccles and Lundberg (195[illegible]) The test stimulation was as a rule submaximal for the group of afferents investigated If not otherwise stated conditioning volleys in the ventral roots were evoked by single [illegible] stimuli maximal for alpha fibres and they preceded the test response by 5—1[illegible] ms [illegible] repetition rate was about 1/s The cerebellar cortex was stimulated with a [illegible] mounted movable ball electrode (cathode) the indifferent electrode being placed in the temporal muscle Rectangular current pulses of 0 2 ms duration and of a strength not exceeding 2 0 [illegible] were used

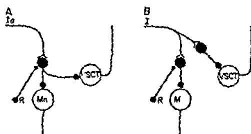


Fig 1 *Schematic representation of two possible alternatives for the organization of the Ia inhibitory pathways to motoneurones (Mn) and VSCT neurones* A inhibition to both types of cells mediated through common interneurones which receive recurrent inhibition through Renshaw cells (R) B inhibition mediated through separate interneurones those *terminating on VSCT cells* lacking inhibition from Renshaw cells Further explanation in text

flexor reflex afferents (FRA) on ascending pathways (Lundberg 1959 1964 Oscarsson 1967 1968 Miller and Oscarsson 1970), among them the VSCT which receives inhibition from the FRA (Oscarsson 1957 1960 Magni and Oscarsson 1961 Eccles *et al* 1961 Lundberg and Weight 1971) Unfortunately the organization of the FRA pathways to motoneurones is still too little known to allow critical testing of this important idea The new VSCT hypothesis has the advantage that it includes one of the simplest and best known interneuronal reflex pathways in the spinal cord the disynaptic reciprocal inhibitory pathway from large muscle spindle (Ia) afferents to motoneurones In fact this pathway has in many ways served as a model for the new VSCT hypothesis

Some VSCT neurones receive monosynaptic excitation and/or disynaptic inhibition from large muscle spindle (Ia) afferents (Lundberg and Weight 1971) One subgroup of these cells has the unique convergence of Ia excitation and inhibition from afferents in the same nerve Lundberg (1971) suggested that the Ia excitation of VSCT cells is related to the excitation of Ia inhibitory interneurones from these afferents and that the corresponding inhibition of the VSCT cells is evoked through collateral connexions from the Ia inhibitory interneurones VSCT cells with Ia excitation and inhibition from the same nerve would thus through collateral connexions receive both the excitatory input and the inhibitory output from the interneurones in the Ia inhibitory pathway to motoneurones Such cells could be considered as input output comparators for this pathway Other Ia excited VSCT neurones may receive only the synaptic effects converging onto the Ia inhibitory interneurones or a fraction of these effects These cells may in a wider sense contribute to the comparator function of the VSCT by providing cerebellum with a reference for the input output comparing cells

The main aim of the present study was to test the important assumption in Lundberg's hypothesis that the Ia inhibition of VSCT neurones is evoked through collateral connexions from the interneurones which relay reciprocal Ia inhibition to motoneurones Our indirect approach has been to compare the pattern of synaptic convergence on the interneurones mediating Ia inhibition to VSCT neurones and to motoneurones, setting out from the finding that the Ia inhibitory interneurones to the latter cells are effectively inhibited from recurrent motor axon collaterals through Renshaw cells (Hultborn *et al* 1971 a b Hultborn 1972 a) If the Ia inhibition of

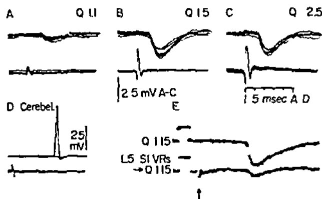


Fig. 2. *Recurrent depression from motor axon collaterals of a Ia IPSP in a VSCT neurone.* Upper traces in A—D are intracellular responses, lower traces are nerve volleys recorded triphasically from the dorsal root entry zone. Positivity is indicated upwards in intracellular recordings and downwards in nerve volley recordings. A—C disynaptic IPSPs evoked by stimulation of Ia afferents in the Q nerve. The strength of the nerve stimulation in multiples of the threshold strength is indicated above the records. D antidromic spike evoked by stimulation of the ipsilateral anterior lobe of cerebellum (Cerebel.). E averaged records of submaximal test Ia IPSP from the Q nerve (upper trace) and test IPSP conditioned by a preceding stimulation of L5—S1 VRs (lower trace). Arrow indicates the arrival of the ventral root volley to the spinal cord. Calibration pulses in E are 1 mV in amplitude and 2 ms in duration. Voltage calibrations in B and D are for intracellular responses.

excitation. It is possible that the present material is more representative than either that of Eccles *et al.* (1961) or that of Lundberg and Weight (1971) with respect to the frequency of occurrence of the synaptic effects listed above, since the microelectrodes usually were inserted from the dorsal column and then traversed both the more dorsal region where Hubbard and Oscarsson (1962) found Ib VSCT cells and the more ventral spinal border cell region where Ia VSCT cells are common (Burke *et al.* 1971, *cf.* also Jankowska and Lindström 1970).

I. Effects from motor axon collaterals on Ia inhibitory pathways to VSCT neurones

a) *Depression of Ia IPSPs*

The conditioning effect of an antidromic volley in motor axons on disynaptic Ia IPSPs in VSCT neurones was tested in 52 cells and a clear depression was found in 43 of these cells. An example of the effect and an illustration of the experimental procedure is given in Fig. 2. The cell was identified as belonging to the VSCT by antidromic invasion, in this case from the anterior lobe of cerebellum (D; *cf.* Methods). The disynaptic IPSP was evoked from the Q nerve and appeared on stimulation of the lowest threshold group I fibres (A). It grew with the first (Ia) component of the group I volley (B) and there was no further increase when the stimulus strength was raised from 1.5 to 2.5 times threshold to activate also the second (Ib) component of the group I volley (C). In this experiment there was a good separation of the Q nerve volley into Ia Ib components as revealed by testing with the double volley technique (Bradley and Eccles 1953; Eccles *et al.* 1957a)—the Ia component being maximal at about 1.5 times threshold which was below

TABLE I Converg nce of monosynaptic EPSPs and disynaptic IPSPs from group I afferents in different nerves onto VSCT neurones with recurrently depressed Ia IPSPs Effects from Ia Ib and group I afferents are indicated by Ia Ib and I respectively Empty spaces indicate no effect IPSPs depressed by a volley in the L5 S1 VRs are marked with +

Cell type	Cell No	Group I EPSPs											
		V	Cr	RF	Sart	Grac	PBSt	ABSm	Add	C S	Pl	FDHL	DP
Q Ia IPSPs	1	Ia		Ia					I				
(largest	2	Ia		Ia					I				
EPSP	3	Ia											
from Q)	4		Ia						Ia				
	5		Ia					I	I				
	6		Ia			I	Ia						
(largest	7					Ia	Ia		I				
EPSP	8					I	Ia		I				
from PBSt	9					I	Ia	I	I				
Add Sart)	10					I	Ia	I	I				
	11						Ia		I				
	12						Ia	Ia					
	13				I	I	Ia	I					
	14								Ia				
	15				Ia								
(without	16												
group I	17												
EPSPs)	18												
	19												
	20												
	21												
	22												
	23												
PBSt Ia	24			Ia									
IPSPs	25		Ia										
(largest	26		Ia										
EPSP	27		Ia		I								
from Q)	28		Ia										
	29		Ia										
	30		Ia										
	31		Ia										

threshold for the Ib component It can therefore be concluded that the IPSP in Fig 1 was evoked from Ia afferents A single volley in the ventral roots L5—S1 preceding the test stimulus by about 8 ms strongly depressed the Ia IPSP as shown by the averaged records in E (upper trace test response lower trace conditioned response) The depres ion of the IPSP was not due to any conductance increase in the VSCT cell membrane caused by the ventral root volley The tiny depolarizing potential in the lower trace of E is a recurrent facilitatory potential (RFP), which is associated with a conductance decrease of the membrane (see section III and Fig 11 C) The RFP should consequently have an opposite effect on the IPSP than that found in Fig 1 E There was no trace of a hidden recurrent IPSP from motor axon collaterals in this or the other cells tested although such IPSPs have been found in a small group of VSCT cells (Lindstrom and Schomburg 1973 a) Since it has

Group I IPSPs										
VCr	RF	Sart	Grac	PBSt	ABSm	Add	G-S	Pl	FDHL	DP
Ia+	Ia+									
Ia+	Ia+			Ib		I	I			
Ia−						I			I	
	Ia+					Ia+			I	
	Ia+						I	I		
	Ia+								I	
Ia+	I								I	
Ia+	I								I	
Ia+	I							I	I	
	Ia+	I						I	I	
	Ia+									
	Ia+						I		I	
	Ia+									
Ia+	I	I					I		I	
	Ia+									
	I +						I	I	I	
	Ia+									
	Ia+						I	I		
	Ia+									
	Ia+								I	
	Ia+									
	Ia+	I								
	Ia+									
				Ia+						
		Ia+		Ia+						
				Ia+						
				Ia+	I	I			I	
				Ia+						
				Ia+	Ia+					
				Ia+						
				Ia+						

earlier been shown that ventral root volleys have no prolonged effect on Ia primary afferents in the cat (*cf* Hultborn *et al* 1971 a) the depression of the Ia IPSP in Fig 1 E must have been due to inhibition exerted at the interneuronal level in the Ia inhibitory pathway

b) *Group I convergence onto cells with depressed Ia IPSPs*

Of the 43 VSCT cells with Ia IPSP susceptible to recurrent depression 36 cells were investigated with respect to the convergence of monosynaptic excitation and disynaptic inhibition from group I muscle afferents in all the muscle nerves listed in the abbreviations Five of these cells received predominant excitation from Ib afferents Since the recurrent control of the Ia inhibitory pathways to Ib VSCT cells will be dealt with in a separate paper (Lindström and Schomburg 1973 b)

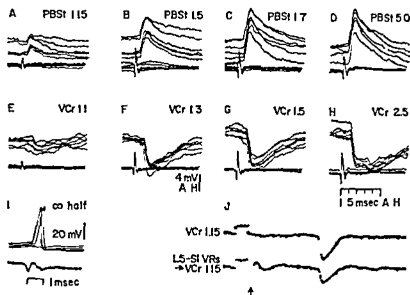


Fig 3 *Depression by a ventral root volley of a Ia IPSP in a VSCT neurone with reciprocally organized Ia excitation and inhibition* A—D monosynaptic EPSPs from Ia afferents in the PBSt nerve E—H disynaptic IPSPs from Ia afferents in the VCr nerve I antidromic spikes evoked by stimulation of the contralateral spinal half (co half) at low thoracic level J test IPSP from the VCr nerve (upper trace) and test IPSP conditioned by stimulation of L5—S1 VRs (lower trace) Averaged records Further details including calibration pulses in J as in Fig 2

these cells will not further be considered The remaining 31 cells are listed in Table I showing the excitatory and inhibitory convergence onto each individual cell Most of these cells received their largest (or only) Ia IPSP from the Q nerve or one of its branches VCr or RF (cells 1—23) The other cells instead had their only or largest Ia IPSP from the PBSt nerve (cells 24—31) Occasionally smaller Ia IPSPs from the Add nerve (in cells with Q Ia IPSPs) or from the Sart or ABSm nerves (in cells with PBSt Ia IPSPs) were tested for recurrent effects and found susceptible to recurrent depression (see Table 1) Of the additional 7 cells in which the group I input from different nerves was incompletely tested but which received Ia IPSPs susceptible to recurrent depression the Ia IPSPs were evoked from the Q nerve in 6 cells two of which also received Ia EPSPs from the Q nerve) and from the PBSt nerve in one cell

When considering the pattern of excitatory input from Ia afferents the cells with Ia IPSPs from the knee extensor Q could be further subdivided into three groups In one group (cells 1—6) the largest Ia excitation was supplied by afferents in the same nerve (Q VCr or RF) In another group (cell 7—14) the excitation and inhibition seemed to be organized reciprocally so that the largest Ia EPSP was evoked from the knee flexors PBSt or Grac or the hip extensor Add (antagonist to RF which has some hip flexor function) One cell with Ia EPSP from the nerve to

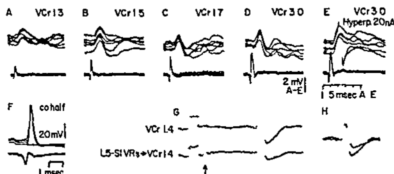


Fig 4 *Depression of a Ia IPSP in a VSCT neurone with convergence of Ia excitation and inhibition from the same nerve* A—D monosynaptic EPSPs and disynaptic IPSPs from Ia afferents in the VCr nerve E as in D but taken during hyperpolarization of the cell by current injection through the recording electrode (20 nA) F antidromic spikes evoked from the contralateral spinal half G test EPSP/IPSP from the VCr nerve (upper trace) and test responses conditioned by stimulation of L5—S1 VRs (lower trace) Averaged records H responses in G superimposed The cell was depolarized with a current of 5 nA when records in G were taken Further details as in Fig 2

Sart (which has some knee flexor function) has also been included in this group (cell 15) The third group with Q Ia IPSPs did not receive group I EPSPs from any of the dissected nerves (cells 16—23) Most of these cells presumably lacked group I excitation also from undissected limb nerves (*cf* Lundberg and Weight 1971)

The cells without monosynaptic group I excitation from any of the dissected nerves are represented by the cell in Fig 2 The records in Fig 3 are from one of the cells with reciprocally organized Ia excitation and inhibition The EPSPs were evoked from Ia afferents in the PBSt nerve (A—D) and the IPSPs from the VCr Ia afferents (E—H) The VCr Ia IPSP was clearly depressed by a ventral root volley (J) Fig 4 gives an example of a cell with Ia excitation and inhibition from the same nerve This unique convergence was noted by Lundberg and Weight (1971) as mentioned in the Introduction However they stimulated the entire Q nerve and although they held it for unlikely the possibility remained that the opposite synaptic effects were from afferents of the VCr (pure knee extensor) and the RF (hip flexor and knee extensors) muscles respectively For this reason the Q nerve was subdivided into the VCr and RF branches in several of the present experiments and as shown in Fig 4 and Table I the convergence of Ia excitation and inhibition was found from either of these branches Both the monosynaptic EPSP and the disynaptic IPSP in the cell of Fig 4 was evoked from the fastest conducting afferents in the Ia component of the VCr nerve volley (A—C) without additional effect when also Ib afferents were stimulated (D) That the late hyperpolarization was a real IPSP is shown by the decrease in amplitude of the potential when the cell was hyper polarized by current injected through the microelectrode (E) Also in this type of cells the Ia IPSPs were depressed by a ventral root volley as shown in G and H (where the two traces in G are superimposed)

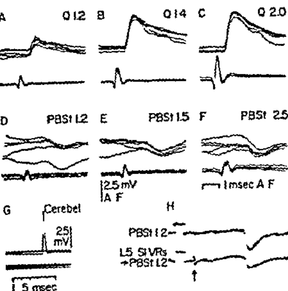


Fig. 5. *Depression of a Ia IPSP from the PBSt nerve in a VSCT neurone.* A—C, monosynaptic Ia EPSPs from the Q nerve. D—F, disynaptic Ia IPSPs from the PBSt nerve. G, antidromic spike evoked from cerebellum. H, test IPSP from the PBSt nerve (upper trace) and test IPSP conditioned by a volley in the L5—S1 VRs (lower trace). Further details as in Fig. 2.

As appears from Table I, cells with Q Ia IPSPs frequently had EPSPs from other nerves than the one supplying the main excitation. These EPSPs were often small compared to the Q or PBSt EPSPs. Several of the cells with Q Ia EPSP/IPSP had small EPSPs from the Add nerve which were most likely evoked from Ia afferents in all cases although listed as group I in Table I. These EPSPs were matched by disynaptic group I IPSPs from the same nerve in 2 cells. In one of them the Add IPSP was clearly evoked from Ia afferents and it was also depressed by a ventral root volley. One cell with a Q Ia EPSP/IPSP received a considerable Ia EPSP from the PBSt nerve and a group I EPSP from the Grac nerve. Cells with PBSt Ia EPSPs received Ia or group I EPSPs from the ABSm, Add and Grac nerves. It is also notable that several cells with Q Ia IPSPs received disynaptic group I IPSPs from the G—S, Pl and FDHL nerves. As will be discussed in section IIa, such IPSPs were probably evoked from Ib afferents and they were unaffected by impulses in motor axons.

The cells with PBSt Ia IPSPs constitute a homogeneous group with respect to the Ia excitation. With the exception of a small additional group I EPSP from the Sart nerve in one cell they received their Ia excitation exclusively from the Q nerve (RF in one cell, see Table I). Fig. 5 shows an example of this type of convergence. In A—C are shown the EPSPs from the Q nerve. The surface electrode was not favourably placed to reveal the Ia/Ib separation in the Q nerve volley when these records were taken, but the Ia volley was maximal at about 1.4 times threshold as revealed by testing with double volley technique and more rostral placement of the surface electrode. The small increase of the EPSP from B to C is presumably due to a contamination of the Ib volley with a small number of Ia fibres. The records in D—F show that the IPSP from the PBSt nerve was evoked from Ia afferents and

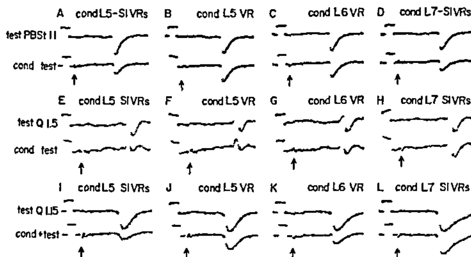


Fig 6 *Effects of volleys in different ventral roots on Ia IPSPs from different nerves* A—D from a cell with Ia IPSP from the PBSt nerve E—H from a cell with Ia EPSP/IPSP from the Q nerve I—L from a cell with Ia IPSP from Q nerve Upper trace in each pair shows the test IPSP evoked by the same strength of stimulation in all records from a given cell Lower traces show the test responses conditioned by a preceding stimulation of different VRs as indicated Arrows indicate the ventral root volleys Averaged intracellular responses The cells in E—H and I—L were identified as belonging to the VSCT by antidromic invasion from cerebellum the cell in A—D by antidromic invasion from the contralateral spinal half All calibration pulses are 1 mV in amplitude and 2 ms in duration

this IPSP was clearly depressed by a ventral root volley (H) A few cells in this group received small group I IPSPs from the Sart or ABSm nerves and two of these IPSPs were found to be depressed by ventral root volleys (Table I) It should be mentioned that although cells with Ia excitation and inhibition from the PBSt nerve were not found in the present study such cells have been described by Lundberg and Weight (1971)

All the IPSPs classified as evoked from Ia afferents and not certainly depressed by a ventral root volley (*cf* section Ia) were evoked from the Q nerve (9 cells) Four of these cells received Q Ia excitation two Q Ib excitation and three cells were without group I excitation from the dissected nerves In VSCT cells with Q Ib excitation there is usually no recurrent effect on Q Ia IPSPs (Lindstrom and Schomburg 1973 b) The negative findings in the other cells may be due to inadequate testing or to erroneous classification of the IPSPs but the possibility that these IPSPs were mediated by Ia inhibitory interneurones not susceptible to recurrent inhibition should not be excluded (*cf* Lindstrom and Schomburg 1973 b)

c) *Effective ventral roots*

The depressive effect on Ia IPSPs described in the previous section was evoked by simultaneous stimulation of all the ventral roots L5 through S1 The individual ventral roots were not equally effective This is illustrated for Ia IPSPs in three different cells in Fig 6 The cell in A—D received its Ia IPSP from the PBSt nerve

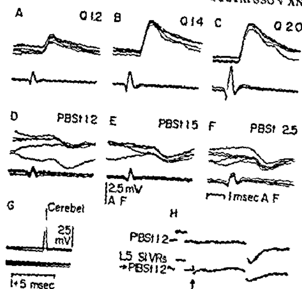


Fig 5 Depression of a Ia IPSP from the PBSt nerve in a VSCT neurone A—C monosynaptic Ia EPSPs from the Q nerve D—F disynaptic Ia IPSPs from the PBSt nerve G antidromic spike evoked from cerebellum H test IPSP from the PBSt nerve (upper trace) and test IPSP conditioned by a volley in the L5—S1 VRs (lower trace) Further details as in Fig 2

As appears from Table I cells with Q Ia IPSPs frequently had EPSPs from other nerves than the one supplying the main excitation These EPSPs were often small compared to the Q or PBSt EPSPs Several of the cells with Q Ia EPSP/IPSP had small EPSPs from the Add nerve which were most likely evoked from Ia afferents in all cases although listed as group I in Table I These EPSPs were matched by disynaptic group I IPSPs from the same nerve in 2 cells In one of them the Add IPSP was clearly evoked from Ia afferents and it was also depressed by a ventral root volley One cell with a Q Ia EPSP/IPSP received a considerable Ia EPSP from the PBSt nerve and a group I EPSP from the Grac nerve Cells with PBSt Ia EPSPs received Ia or group I EPSPs from the ABSm Add and Grac nerves It is also notable that several cells with Q Ia IPSPs received disynaptic group I IPSPs from the G—S Pl and FDHL nerves As will be discussed in section IIa such IPSPs were probably evoked from Ib afferents and they were unaffected by impulses in motor axons

The cells with PBSt Ia IPSPs constitute a homogeneous group with respect to the Ia excitation With the exception of a small additional group I EPSP from the Sart nerve in one cell they received their Ia excitation exclusively from the Q nerve (RF in one cell see Table I) Fig 5 shows an example of this type of convergence In A—C are shown the EPSPs from the Q nerve The surface electrode was not favourably placed to reveal the Ia/Ib separation in the Q nerve volley when these records were taken but the Ia volley was maximal at about 1.4 times threshold as revealed by testing with double volley technique and more rostral placement of the surface electrode The small increase of the EPSP from B to C is presumably due to a contamination of the Ib volley with a small number of Ia fibres The records in D—F show that the IPSP from the PBSt nerve was evoked from Ia afferents and

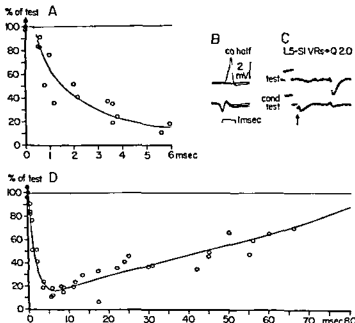


Fig 7 *Time course of the depression by ventral root volleys of Ia IPSPs in a VSCT neurone* B antidromic spike evoked in the cell from the contralateral spinal half C an example of the test Ia IPSP from the Q nerve (upper trace) and the test IPSP conditioned by stimulation of the L5—S1 VRs (lower trace) averaged records In the graphs A and D the amplitudes of the conditioned IPSPs expressed as a percentage of the unconditioned test IPSPs (ordinates) are plotted against the conditioning testing intervals (abscissae) Each point in the graphs is derived from a pair of averaged records as in C The time intervals were measured between the arrival to the spinal cord of the ventral root volley and the Q volley as recorded with the cord surface electrode simultaneously with the averaging Further details as in Fig 2

stimulation due to recurrent inhibition of the motoneurones there remained the possibility that this negative result was caused by the use of wrong ventral roots Accordingly it was of interest to test the effects of ventral root stimulation on Ib IPSPs evoked in VSCT neurones (Eccles *et al* 1961 Lundberg and Weight 1971) which only occasionally receive recurrent inhibition (Lindstrom and Schomburg 1973 a) Disynaptic Ib IPSPs are evoked in all three main categories of VSCT cells (with Ia Ib or no group I excitation) most frequently from the Q nerve In addition many VSCT cells receive disynaptic group I IPSPs from nerves which do not display group Ia/Ib separation Such IPSPs are often evoked from the nerves to the ankle and toe extensors G—S Pl and FDHL Confirming Lundberg and Weight (1971) we observed that IPSPs from the latter nerves usually increase in the high threshold group I range They are therefore presumably in most cases evoked from Ib afferents VSCT cells also receive IPSPs which grow within the Ib range and have segmental latencies indicating two or more interposed interneurones in the pathway (Eccles *et al* 1961 Lundberg and Weight 1971) Also these IPSPs are most frequently evoked from the Q nerve

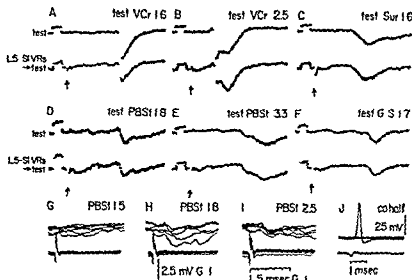


Fig 8 *Effects on IPSPs from different groups of afferents in a VSCT neurone* A—F averaged intracellular responses Upper traces in records A—F show test IPSPs evoked from different groups of afferents A group Ia afferents in the VCr nerve B group Ia and Ib afferents in the VCr nerve C low threshold cutaneous afferents in the Sur nerve D group Ib afferents in the PBSt nerve E group Ib and II afferents in the PBSt nerve F group I afferents in the G—S nerve. Lower traces show the IPSPs conditioned by an antidromic volley in the L5—S1 VRs G—I effects of graded stimulation of the PBSt nerve showing the Ib origin of the disynaptic IPSP Stimulation strength maximal for Ia afferents in G and maximal for group I afferents in I J antidromic spike evoked in the cell from the contralateral spinal half Further details in Fig 2 The cell was depolarized by current injection (40 nA) through the recording electrode when records in A—I were taken

The effects of impulses in motor axons were tested on all the above three types of IPSP Disynaptic Ib IPSPs from the Q PBSt or Add nerves were tested in 17 cells group I IPSPs from G—S Pl or FDHL nerves in 22 cells and group I IPSPs from other nerves in 9 cells Polysynaptic Ib IPSP were investigated in 28 cells In several cells IPSPs were tested from more than one nerve With the exception of a group I IPSP from the G—S nerve in one cell which seemed to be slightly depressed all these IPSP were unaffected by antidromic volleys in the L5—S1 VRs This was the case also in cells which received Ia IPSPs susceptible to recurrent depression as illustrated in Fig 8 This cell received a Ia IPSP from the Q nerve which was clearly depressed by the ventral root stimulation (A) From the same nerve a late polysynaptic IPSP appeared at a stimulation strength of about 1 6 times threshold for the Ia fibres This IPSP is an example of a presumed polysynaptic Ib IPSP and B shows that it was unaffected by the ventral root volley At higher stimulation strength there appeared a still later group II IPSP which was also unaffected by the ventral root volley (not illustrated) The same cell also received a small disynaptic Ib IPSP from the PBSt nerve as shown by the records in G—I and a group I IPSP from the

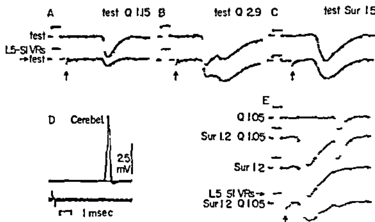


Fig 9 *Convergence of facilitation from low threshold cutaneous afferents and of inhibition from motor axon collaterals in the Ia inhibitory pathway to a VSCT neurone* A—C and E are averaged intracellular responses Upper traces in A—C show test IPSPs evoked from group Ia afferents in the Q nerve (A) from group Ia and II afferent in the Q nerve (B) and from low threshold cutaneous afferents in the Sur nerve (C) lower traces show the IPSP conditioned by an antidromic volley in the L5—S1 VRs The traces in E show from the top to the bottom a test Ia IPSP from the Q nerve facilitation of this test IPSP by a preceding stimulation of the Sur nerve the Sur IPSP alone and the Sur facilitated Ia IPSP conditioned by a preceding stimulation of the L5—S1 VRs (arrow) D antidromic spike evoked from cerebellum. Further details as in Fig 2

G—S nerve Both these IPSPs were likewise unaffected by the ventral root stimulation (D and F) With respect to the lack of effect on Ib IPSPs the results from VSCT cells thus correspond to the results from motoneurones

b) *Effects on IPSPs from flexor reflex afferents (FRA)*

When considering their spinal effects group II and III muscle afferents cutaneous afferents and high threshold joint afferents are often grouped together as flexor reflex afferents (FRA R M Eccles and Lundberg 1959 Holmqvist and Lundberg 1961) In some motoneurones IPSPs from such afferents were slightly depressed by ventral root stimulation (Hultborn *et al* 1971 a) This finding was assumed to be due to excitation of the Ia inhibitory interneurones from these afferents thereby giving FRA IPSPs which partly were evoked via the Ia inhibitory interneurones and thus susceptible to recurrent depression This interpretation has been supported by the results of a recent study showing a striking correlation between the occurrence of facilitation of Ia IPSPs in motoneurones from the FRA and of recurrent depression of FRA IPSPs in the same cells (Fedina and Hultborn 1972)

Since practically all VSCT neurones receive polysynaptic FRA IPSPs (Eccles *et al* 1961 Lundberg and Weight 1971) it was of interest to compare the effects of ventral root stimulation on FRA IPSPs in cells with and without Ia IPSPs The test IPSPs were evoked from both ipsi and contralateral group II and III muscle afferents and cutaneous afferents Usually IPSPs from more than one nerve were tested in each

cell and all dissected nerves (*cf* Abbreviations) were tried the Q G—S Sur CoH and CoSur nerves being used most frequently In 63 cells without Ia IPSPs the FRA IPSPs were unaffected by ventral root stimulation while FRA IPSPs in 13 of 29 cells with recurrently depressed Ia IPSPs were clearly susceptible to recurrent depression As in motoneurones not all the FRA IPSPs were decreased in these cells but affected FRA IPSPs *included IPSPs from both ipsi and contralateral cutaneous and muscle* afferents The findings with respect to FRA IPSPs are illustrated in Figs 8 and 9 In spite of a clear depression of the Ia IPSP in the cell of Fig 8 there was no effect on IPSPs from cutaneous nerves or group II muscle afferents as exemplified by the Sur IPSP in C and the late IPSP from the PBSt nerve in E On the other hand the IPSP from the Sur nerve was clearly depressed in the cell of Fig 9 (C) In the same cell the group II IPSP from the Q nerve seems to be unaffected (late peak in B)

In motoneurones the ventral root effect on FRA IPSPs was parallelled by a facilitatory effect from these efferents on Ia IPSPs (Fedina and Hultborn 1972) A similar facilitation was found in tested VSCT cells as shown in Fig 9 E The upper trace shows the small test Ia IPSP which was clearly facilitated by a submaximal Sur volley (second trace)—the Sur IPSP alone is shown in the third trace When preceded by a ventral root volley (lowermost trace) the Sur IPSP is depressed and the facilitated Ia IPSP is virtually abolished Since the ventral root depression of the facilitated Ia IPSP is larger than the test IPSP alone all these effects must have converged onto common Ia inhibitory interneurones Effects similar to those in Fig 9 were found with respect to 7 FRA IPSPs tested in 4 other cells The tests included IPSPs evoked from both ipsi and contralateral high threshold muscle afferents

It should be mentioned that several of the VSCT cells with Ia IPSPs susceptible to recurrent depression also received initial FRA excitation from one or more nerves. This was especially notable in cells with convergence of Q Ia EPSPs/IPSPs half of which received such an excitation from several nerves

c) Effects on IPSPs from descending tracts

VSCT cells receive mono- di and polysynaptic IPSPs from several ipsilateral descending tracts (Oscarsson 1965 Baldissera and Bruggencate 1969 Baldissera and Weight 1969) Disynaptic IPSPs are of special interest in this connexion since Ia inhibitory interneurones to motoneurones receive monosynaptic excitation from descending tracts (Grillner *et al* 1966 Grillner and Hongo 1972 Jankowska *et al* 1973) No attempt was made to study recurrent effects on descending IPSPs identified with respect to the activated descending tract However in 9 cells (8 with Ia IPSPs and 1 with disynaptic Ib IPSP) disynaptic IPSPs evoked by stimulation of the ipsilateral spinal half at low thoracic level (except the dorsal funiculus) was conditioned by a preceding ventral root stimulation In the cell with Ib IPSP there was no effect but in 7 of the remaining 8 cells there was a clear depression of the IPSP Affected IPSPs were found in cells with Ia IPSPs from either the Q or PBSt nerves An example of the effect is shown in Fig 10 In B is a large descending IPSP of which at least the initial part is evoked disynaptically (segmental latency 1.3 ms

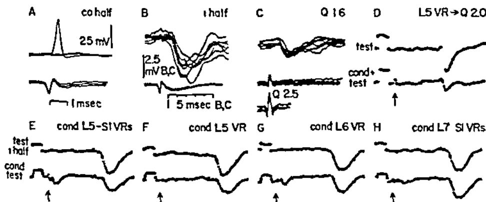


Fig 10 *Depression of a disynaptic descending IPSP in a VSCT neurone with a Ia IPSP* A antidromic spike from contralateral spinal half B disynaptic IPSP evoked by stimulation of the ipsilateral spinal half (except the dorsal column) at low thoracic level C Ia IPSP from the Q nerve (lowermost trace shows maximal Q group I incoming volley) D—H are averaged intracellular responses upper traces show test IPSPs from the Q nerve (D) and ipsilateral spinal half (E—H) lower traces show the IPSPs conditioned by volleys in indicated VRs Further details as in Fig 2

as measured from the first positive peak of the descending volley) This initial part of the IPSP is clearly depressed by the ventral root volley (E) The same cell received its Ia IPSP from the Q nerve (C D) and it is therefore interesting that the descending IPSP was depressed from the L5 and L6 VRs (F G) but not from the L7—S1 VRs (H) similarly to the Q Ia IPSP in the cell (not illustrated) This indicates that the descending IPSP was evoked through the Ia inhibitory interneurones terminating on the VSCT cell

Monosynaptic descending IPSPs tested in a few cells were unaffected by the ventral root volley Polysynaptic descending IPSPs were not tested

III Recurrent facilitation of VSCT neurones from motor axon collaterals

In unanesthetized preparations recurrent facilitatory potentials (RFPs) are evoked in motoneurones from certain motor axons (Wilson and Burgess 1962 Hultborn *et al* 1971 d) These recurrent facilitatory potentials are due to disinhibition of the motoneurones (Wilson *et al* 1960 Wilson and Burgess 1962 Hultborn *et al* 1971 d) and are associated with a decreased conductance in the motoneuronal membrane (Hultborn *et al* 1971 d) The main source of the tonic inhibition removed during recurrent facilitation of motoneurones is most likely the Ia inhibitory interneurones which have a resting discharge in unanesthetized preparations (Hultborn *et al* 1971 d) Another possible source of this tonic inhibition are Renshaw cell which also receive recurrent inhibition (Ryall 1970)

Recurrent facilitatory potentials were looked for in 18 VSCT cells recorded in unanesthetized cats (see Methods) RFPs were found in 8 of these cells 7 of which received disynaptic Ia IPSPs from some of the dissected nerves. None of the cells without RFPs received Ia IPSPs. Small RFPs were in addition found in 6 other cells recorded in anesthetized preparations all with disynaptic Ia IPSPs Cells with RFPs showed no trace of recurrent IPSPs although such effects have been found in some VSCT neurones (Lindström and Schomburg 1973 a) The RFPs in the VSCT cells hade segmental latencies of 1.9—2 6 ms and durations around 40—60 ms which are within the ranges of corresponding values in motoneurones (Wilson and Burgess 1962 Hultborn *et al* 1971 d) Examples of RFPs in a VSCT cell are shown in Fig 11 B—C The RFPs were evoked by a volley in the L5—L6 VRs which also effectively depressed the Ia IPSP in the cell (B) A volley in the L7—S1 VRs did not evoke any RFP and was likewise without effect on the Ia IPSP (D) Also in VSCT cells the RFPs are associated with a decreased conductance in the cell membrane This is shown in C by the increase in amplitude of a monosynaptic test EPSP from the PBSt nerve placed on top of the RFP The RFPs in motoneurones are very susceptible to barbiturate anesthesia presumably because the Ia inhibitory interneurones cease to fire tonically in anesthetized preparations (Hultborn *et al* 1971 d) The same is true also for RFPs in VSCT neurones as shown in Fig 10 E These records were taken from the same cell as B—C but a few minutes after iv injection of pentobarbitone sodium (3 mg/kg)

The recurrent facilitation of VSCT neurones could be detected also on the VSCT mass discharge recorded from a dissected ventral quadrant on the contralateral right side of the spinal cord at the level of Th12 Fig 11 F shows the time course of the recurrent facilitation of VSCT cells determined by conditioning a maximal group I VSCT mass discharge evoked from the Q nerve by a single volley in the L5—S1 VRs By grading the strength of stimulation of the Q nerve it was found that the facilitation was exerted on VSCT neurones excited from group Ia afferents (*cf* Lindström and Schomburg 1973 a) Similar effects have been observed on VSCT discharges evoked from the PBSt nerve

Discussion

The present results unambiguously demonstrate that disynaptic IPSPs evoked from large muscle spindle Ia afferents in VSCT neurones can be depressed by impulses in motor axons as is the case with disynaptic Ia IPSPs in motoneurones (Hultborn *et al* 1971 a The depression occurred without recurrent inhibitory effects on the VSCT cell membrane Since antidromic impulses in motor axons do not give rise to any prolonged presynaptic effects in Ia primary afferents in the cat (*cf* Hultborn *et al* 1971 a) it is concluded that the recurrent effect was exerted on the interneurones interposed in the pathway The segmental latency and the time course of the effect were similar to those in motoneurones which indicates that the depression was due to postsynaptic inhibition of the Ia inhibitory interneurones mediated

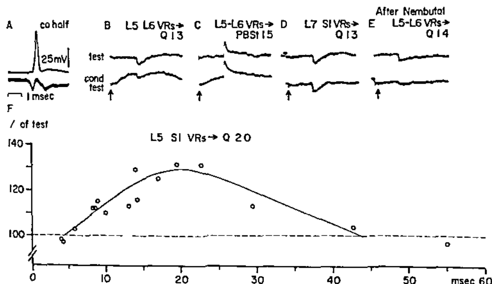


Fig 11 *Recurrent facilitation from motor axon collaterals of VSCT neurones* A—E show intracellular responses from a VSCT neurone A antidromic spike evoked from the contralateral spinal half Upper traces in B—E show test responses disynaptic Ia IPSPs from the Q nerve (B D E) and monosynaptic Ia EPSP from the PBSt nerve (C) lower traces in B—E show effects of an antidromic volley in the indicated ventral roots The records in E were taken a few minutes after Nembutal (3 mg/kg) was given i v Calibration pulses in B—E are 0 5 mV in amplitude and 1 ms in duration The graph in F shows the time course of recurrent facilitation of a VSCT mass discharge by a volley in the L5—S1 VRs The VSCT discharge was evoked by a group I volley in the Q nerve and was recorded from a contralateral ventral quadrant of the spinal cord at low thoracic level The amplitudes of the conditioned VSCT discharge expressed as a percentage of the unconditioned test discharge are plotted against the conditioning testing intervals determined as in Fig 7 Each point in the graph represents the average amplitude of 128 responses Further details as in Fig 2

disynaptically from motor axon collaterals through Renshaw cells (*cf* Renshaw 1941 Eccles *et al* 1954 Hultborn *et al* 1971 a) Further Ia IPSPs from a given nerve were depressed from the same ventral roots as the Ia IPSP evoked from this nerve in motoneurones Thus Q Ia IPSPs were depressed from the L5 and L6 VRs but not from the L7—S1 VRs while PBSt IPSPs were depressed mainly from the L7—S1 VRs and never from the L5 VR In motoneurones this distribution of effective ventral roots is a consequence of the rostrocaudal distribution of different motor nuclei (Q in L5—L6 and PBSt in L7—S1) and the fact that the main inhibition of the Ia inhibitory interneurones is supplied by efferents muscle as the one supplying Ia excitation to the interneurones (1971 c) The same principle seems to be valid also for Ia inh terminating on VSCT cells The recurrent control of the Ia inh VSCT neurones seems thus to be similarly organized as the re corresponding pathway to motor

Also with respect to recurrent ff IPSPs r present results fully correspond to in

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cells with Ia IPSPs Since the descending IPSPs were depressed by the same ventral root volleys as the Ia IPSPs in the cells it is likely that also these IPSPs were mediated by Ia inhibitory interneurones terminating on the VSCT cells In this connexion it should be mentioned that the rubrospinal tract which mainly through a polysynaptic linkage facilitates the transmission in the Ia inhibitory path to motoneurones al o is known to facilitate the Ia inhibitory path to VSCT neurones (Baldissera and Bruggencate unpublished)

Recurrent facilitation of motoneurones is due to removal of a tonic inhibitory input caused by recurrent inhibition of the interneurones which supply this tonic inhibition (Wilson *et al* 1960 Wilson and Burgess 1962 Hultborn *et al* 1971 d) The main source of the tonic inhibition in motoneurones seems to be the Ia inhibitory interneurones (Hultborn *et al* 1971 d) which have a considerable resting activity in unanesthetized preparations Renshaw cells which also receive recurrent inhibition (Ryall 1970) seem to be of minor importance RFPs with the same segmental latency and time course as RFPs in motoneurones were evoked also in VSCT cells in unanesthetized preparations and the RFPs were as sensitive to barbiturate anesthesia as RFPs in motoneurones With a single exception the RFPs were evoked in cells which received Ia IPSPs and tested cells without RFPs also lacked Ia IPSP The exceptional cell presumably received Ia IPSPs from some un dissected nerve None of these VSCT cells received recurrent inhibition (*cf* Lindström and Schomburg 1973 a) Therefore recurrent facilitation also in VSCT neurones seems to be related to the Ia inhibitory pathway This conclusion is further emphasized by the finding that RFPs were evoked only from those ventral roots which depressed Ia IPSPs in the same cell

All the recurrent effects from motor axon collaterals or transmission to VSCT neurones are thus explained by a selective action on the interneurones transmitting Ia inhibition to the VSCT When considering the significance of the fact that recurrent inhibition is exerted on Ia inhibitory interneurones projecting to VSCT neurones and to motoneurones it is important to note that susceptibility to recurrent inhibition from motor axon collaterals is not a general property of Ia inhibitory interneurones For example dorsal spinocerebellar tract neurones (Lindstrom and Takata to be published) some spinal interneurones (Lindstrom and Schomburg unpublished) and even a special group of VSCT neurones with Ib excitation (Lind strom and Schomburg 1973 b) receive disynaptic Ia IPSPs which are unaffected by impulses in motor axons Clearly the most simple explanation of the parallel recurrent effects onto Ia IPSPs in VSCT neurones and motoneurones would be that the same Ia inhibitory interneurones project to both groups of cells The finding that Ia IPSPs from a given nerve both in VSCT cells and motoneurones are depressed from the same ventral roots gives additional support for the idea of a common interneuronal origin of these IPSPs

As discussed above there is also in other respects a striking correspondence between the organization of the Ia inhibitory pathways to VSCT neurones and motoneurones For example the subsidiary effects from the FRA are similar and the Ia inter

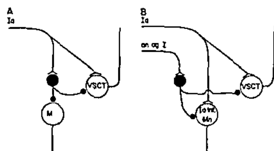


Fig 12 *Schematic representation of presumed pathways mediating Ia excitation and inhibition to two different types of VSCT neurones* The VSCT cell in A receives collateral connexion both from the interneurones in the Ia inhibitory pathway to motoneurones (Mn) and from the Ia afferents impinging on these interneurones The VSCT cell in B receives through collateral connexions Ia excitation and inhibition representing the reciprocally organized input to Ia inhibitory interneurones (Ia int) or motoneurones (Mn) The target Ia inhibitory interneurones and motoneurones are for simplicity drawn as a single cell (hatched) in the diagram Further explanation in text

neurones terminating on VSCT cells and motoneurones have a similar resting activity in unanesthetized preparations with the same high sensitivity to barbiturate anesthesia There seems also to be a close parallelism between the descending excitatory control of the two Ia inhibitory pathways All these similarities in the control of the Ia inhibitory pathways to motoneurones and VSCT neurones are to be expected from the hypothesis of common inhibitory interneurones (*cf* Introduction) The alternative possibility with two parallel Ia inhibitory pathways with practically identical convergence on the interneurones is indeed unlikely On the basis of the indirect evidence summarized above it is therefore concluded that *Ia IPSPs in many VSCT neurones are evoked through collateral connexions from interneurones which mediate Ia reciprocal inhibition to motoneurones*

The existence of collateral connexions to VSCT cells from the interneurones in the reciprocal Ia inhibitory pathway to motoneurones enables the VSCT to signal information about this pathway The possible mechanisms for this information r have been discussed extensively (Lundberg and Weight 1970 Lundberg 1971 Lindstrom and Schomburg 1973 a) and will be considered further in another context (Lindstrom 1973) Here will only be pointed out that Ia IPSPs susceptible to recurrent depression were found in VSCT neurones with different types of convergence from Ia afferents (*cf* section I b) This is important since the Ia inhibition of the VSCT neurones has been given different significance depending on the type of convergence of Ia excitation onto the cells (Lundberg 1971) In cells with Ia excitation and inhibition from afferents in the same nerve the inhibition has been considered to represent the output from Ia inhibitory interneurones (Fig 12 A) In other VSCT neurones with mainly reciprocally organized Ia excitation and inhibition the Ia inhibition may represent a part of the input to Ia inhibitory interneurones or motoneurones (Fig 12 B) (Note that the interneurones which mediate Ia reciprocal inhibition to motoneurones do themselves receive a similar reciprocally organized Ia inhibition Hultborn 1972 b) In cells without group I excitation the inhibition may play either of these roles (*cf* Lundberg 1971) These ideas are fully compatible with the present findings that Ia IPSPs in VSCT cells with all three types of convergence are susceptible to recurrent depression from motor axon collaterals

There is a good agreement between the overall pattern of convergence of group I excitation and inhibition from different nerves onto VSCT neurones in this and earlier studies (cf Results) When considering the detailed pattern of connexions to individual VSCT neurones it should be realized that the present sample is small and all cells may not be representative Too much importance should probably therefore not be attached to the finding that the convergence in some cells (for instance cell 6 Table I) differs from what would be expected if the effects were exclusively supplied by collateral connexions from neurones terminating on typical Ia inhibitory interneurones or motoneurones (cf R M Eccles and Lundberg 1958 Hultborn and Udo 1972 b) Unusual patterns of convergence are occasionally found also in individual motoneurones (Eccles *et al* 1957 b) or Ia inhibitory interneurones (Hultborn *et al* 1971 b Lindstrom and Schomburg unpublished) It is also possible that some VSCT neurones convey a highly integrated message regarding different reflex pathways (cf Lundberg 1971 Multiplicity hypothesis) With respect to the convergence of group I IPSPs from the G-S Pl or FDHL nerves in the VSCT neurones it might be of importance that disynaptic group I IPSPs from the FDHL nerve have been observed in some recurrently inhibited Ia interneurones (Lindstrom and Schomburg unpublished) The frequent occurrence of early FRA excitation in VSCT neurones with recurrently depressed Ia IPSPs may be of relevance in relation to the FRA excitation of the Ia inhibitory interneurones

The present results concerning the Ia inhibitory pathway and the recent finding of recurrent inhibition to some VSCT neurones from motor axon collaterals (Lindstrom and Schomburg 1973 a) strongly support the hypothesis that the VSCT conveys information about the complex integration at interneuronal level in segmental reflex pathways to motoneurones (Lundberg 1971) Thus the VSCT may function as a central feed back pathway rather than as a relay of afferent information—the input to the VSCT from the periphery being a consequence of the afferent input from peripheral receptors to segmental interneurones which are also governed from higher centres The finding that rhythmic modulation of the discharges in VSCT neurones in walking cats remains after deafferentation (Arshavsky *et al* 1972) gives additional support to the idea that the VSCT relays information about central events

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References

Andén N E M G M Jukes A Lundberg and L Vyklický Th effe t of DOPA on the spinal cord 1 Influence on transmission f om p imary affe ents A ta ph| l and 1966 67 373—386

Arshavsky Y I M B Berkinblit O I Fukson I M Gelfand and G N Orlovsky Origin of modulation in neurones of the ventral pinocerebellar t act during locomot on B a n Res 1972 43 276—279

Baldissera F and G ten Bruggencate R b o p nal effects on spinal border cells A ta physiol scand 1969 Suppl 330 119

Baldissera F and F Weight Descending monosynapt c connexions to p nal bo der cells A ta physiol scand 1969 76 28 —294

Bradley K and J C Eccles Analysis of the fast afferent impulses f om thigh muscles J Physiol (Lond) 1953 122 462—473

Burke R A Lundberg and F Weight Spinal border cell orig n of the ventral spinocerebella tract Exp Brain Res 1971 12 283—294

ECCLES R M and A LUNDBERG Integrative patterns of Ia synaptic actions on motoneurones of hip and knee muscles *J Physiol* (Lond) 1958 *144* 271—298
ECCLES R M and A LUNDBERG Synaptic actions in motoneurones by afferents which may evoke the flexion reflex *Arch ital Biol* 1959 *97* 199—221
ECCLES J C R M ECCLES and A LUNDBERG Synaptic actions on motoneurones in relation to the two components of the group I muscle afferent volley *J Physiol* (Lond) 1957 a *136* 527—546
ECCLES J C R M ECCLES and A LUNDBERG The convergence of monosynaptic excitatory afferents on to many different species of α motoneurones *J Physiol* (Lond) 1957 b *137* 22—50
ECCLES J C R M ECCLES and A LUNDBERG Synaptic actions on motoneurones caused by impulses in Golgi tendon organ afferents *J Physiol* (Lond) 1957 c *138* 227—252
ECCLES J C P FATT and K KOKETSU Cholinergic and inhibitory synapses in a pathway from motor axon collaterals to motoneurones *J Physiol* (Lond) 1954 *126* 524—562
ECCLES J C J I HUBBARD and O OSCARSSON Intracellular recording from cells of the ventral spino-cerebellar tract *J Physiol* (Lond) 1961 *158* 486—516
FEDINA L and H HULTBORN Facilitation from ipsilateral primary afferent of interneuronal transmission in the Ia inhibitory pathway to motoneurones *Acta physiol scand* 1972 *86* 59—81
GRILLNER S and T HONGO Vestibulospinal relations Vestibular influences on the lumbosacral spinal cord In *Basic Aspects of Central Vestibular Mechanisms* Ed A Brodal and O Pompeiano Elsevier Publ Co 1972
GRILLNER S T HONGO and S LUND Interaction between the inhibitory pathways from the Deiter's nucleus and Ia afferents to flexor motoneurones *Acta physiol scand* 1966 *68* Suppl 277 61
GUSTAFSSON B and S LINDSTRÖM Depression of Ia IPSPs in spinal border cells by impulses in recurrent motor axon collaterals *Acta physiol scand* 1970 *80* 13A—14A
HOLMQVIST B and A LUNDBERG Differential supraspinal control of synaptic actions evoked by volleys in the flexion reflex afferents in alpha motoneurones *Acta physiol scand* 1961 *54* Suppl 186 1—51
HONGO T E JANKOWSKA and A LUNDBERG The rubrospinal tract IV Effects on interneurones *Exp Brain Res* 1972 *15* 54—78
HUBBARD J I and O OSCARSSON Localization of the cell bodies of the ventral spino-cerebellar tract in lumbar segments of the cat *J comp Neurol* 1962 *118* 199—204
HULTBORN H Convergence on interneurones in the reciprocal Ia inhibitory pathway to motoneurones *Acta physiol scand* 1972 a Suppl 375
HULTBORN H *On the control of transmission in the reciprocal Ia inhibitory pathway to motoneurones* In *Mechanisms of neuronal integration in nerve centers* Kiev 1972 b
HULTBORN H and M SANTINI Supraspinal control of monosynaptically activated group Ia interneurones in the ventral horn *Acta physiol scand* 1972 *84* 142—144
HULTBORN H and M UDO Convergence in the reciprocal Ia inhibitory pathway of excitation from descending pathways and inhibition from motor axon collaterals *Acta physiol scand* 1972 a *84* 95—108
HULTBORN H and M UDO Convergence of large muscle spindle (Ia) afferents at interneuronal level in the reciprocal Ia inhibitory pathway to motoneurones *Acta physiol scand* 19[illegible] b *84* 493—499
HULTBORN H and M UDO Recurrent depression from motor axon collaterals of supraspinal inhibition in motoneurones *Acta physiol scand* 1972 c *85* 44—57
HULTBORN H E JANKOWSKA and S LINDSTROM Recurrent inhibition from motor axon collaterals of transmission in the Ia inhibitory pathway to motoneurones *J Physiol* (Lond) 1971 a *15* 591—61[illegible]
HULTBORN H E JANKOWSKA and S LINDSTROM Recurrent inhibition of interneurones monosynaptically activated from group Ia afferents *J Physiol* (Lond) 1971 b *215* 613—636
HULTBORN H E JANKOWSKA and S LINDSTROM Relative contribution from different nerves to recurrent depression of Ia IPSPs in motoneurones *J Physiol* (Lond) 1971 c *215* 637—664
HULTBORN H E JANKOWSKA S LINDSTROM and W ROBERTS Neuronal pathway of the recurrent facilitation of motoneurones *J Physiol* (Lond) 1971 d *218* 495—514
JANKOWSKA E and S LINDSTROM Morphological identification of physiologically defined neurones in the cat spinal cord *Brain Res* 1970 *20* 323—3[illegible]
JANKOWSKA E A LUNDBERG and D STUART Propriospinal control of last order interneurones of spinal reflex pathways in the cat *Brain Res* 1973 In press

LINDSTROM S Recurrent control from motor axon collaterals of Ia inhibitory pathways in the spinal cord of the cat *Acta physiol scand* 1973 Suppl 392
LINDSTROM S and E D SCHOMBURG Recurrent inhibition from motor axon collaterals of ventral spinocerebellar tract neurones *Acta physiol scand* 1973 a *88* 505—515
LINDSTROM S and E D SCHOMBURG Group I inhibition in Ib excited ventral spinocerebellar tract neurones *Acta physiol scand* 1974 b In press
LUNDBERG A Integrative significance of patterns of connections made by muscle afferents in the spinal cord *Symp XXI Int Physiol Congr* Buenos Aires 1959 1—5
LUNDBERG A Ascending spinal hindlimb pathways in the cat In *Progress in Brain Research* Eds J C Eccles and J P Schade *Physiology of spinal neurons* Amsterdam Elsevier Publ Co 1964 12 135—163
LUNDBERG A Integration in the reflex pathway In *Muscular Afferents and Motor Control* Nobel Symposium I Ed. R Granit Almqvist & Wiksell Stockholm 1966 275—305
LUNDBERG A Convergence of excitatory and inhibitory action on interneurones in the spinal cord In *The Interneuron* Ed M A B Brazier UCLA Forum Med Sci No 11 University of California Press Los Angeles 1969 231—265
LUNDBERG A Function of the ventral spinocerebellar tract—a new hypothesis *Exp Brain Res* 1971 *12* 317—330
LUNDBERG A and O OSCARSSON Functional organization of the ventral spinocerebellar tract in the cat IV Identification of units by antidromic activation from the cerebellar cortex *Acta physiol scand* 1962 *54* 252—269
LUNDBERG A and P VOORHOEVE Effects from the pyramidal tract on spinal reflex arcs *Acta physiol scand* 1962 *56* 201—219
LUNDBERG A and F WEIGHT Signalling of reciprocal Ia inhibition by the ventral spinocerebellar tract *Brain Res* 1970 *23* 109—111
LUNDBERG A and F WEIGHT Functional organization of connexions to the ventral spinocerebellar tract *Exp Brain Res* 1971 *12* 295—316
MAGNI F and O OSCARSSON Cerebral control of transmission to the ventral spinocerebellar tract. *Arch ital Biol* 1961 *99* 369—396
MILLER S and O OSCARSSON Termination and functional organization of spino olivocerebellar paths In *The Cerebellum in Health and Disease* Ed W S Fields and W D Willis Warren H Green St Louis 1970 172—200
OSCARSSON O Functional organization of the ventral spino-cerebellar tract in the cat II Connections with muscle joint and skin nerve afferents and effects on adequate stimulation of various receptors *Acta physiol scand* 1957 *42* Suppl 146 1—10
OSCARSSON O Functional organization of the ventral spino-cerebellar tract in the cat III Supraspinal control of VSCT units of I type *Acta physiol scand* 1960 *49* 171—183
OSCARSSON O Functional organization of the spinocerebellar tracts *Physiol Rev* 1965 *45* 495—522
OSCARSSON O Functional significance of information channels from the spinal cord to the cerebellum In *Neurophysiological Basis of Normal and Abnormal Motor Activities* 3rd Symposium of the Parkinson's Disease Information and Research Center Ed M D Yahr and D P Purpura Raven Press Hewlett N Y 1967 93—117
OSCARSSON O Termination and functional organization of the ventral spino-olivocerebellar path *J Physiol* (Lond) 1968 *196* 453—478
RENSHAW B Influence of discharge of motoneurons upon excitation of neighboring motoneurons *J Neurophysiol* 1941 *4* 167—183
RYALL R W Renshaw cell mediated inhibition of Renshaw cells Patterns of excitation and inhibition from impulses in motor axon collaterals *J Neurophysiol* 1970 *33* 257—270
WILSON V J and P R BURGESS Disinhibition in the cat spinal cord *J Neurophysiol* 1962 *25* 392—404
WILSON V J F P J DIECKE and W H TALBOT Action of tetanus toxin on conditioning of spinal motoneurons *J Neurophysiol* 1960 *23* 659—666

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Contraction Properties of Cat Facial Muscles

By

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Abstract

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The contraction properties of some facial muscles of the cat have been investigated The contraction time of a maximal isometric twitch is 8 5 ms for the orbicularis oculi 24 ms for the depressor conchae and 33 ms for the orbicularis oris It is suggested that a slower decay of the active state may be one factor explaining the longer contraction time of the orbicularis oris as compared to the orbicularis oculi The duration of a maximal compound action potential is also longer for the orbicularis oris than for the orbicularis oculi (8 5 and 2 8 ms respectively) Muscle length is more critical for the twitch amplitude of the orbicularis oculi than for the orbicularis oris Summation of individual twitches occurs at a stimulus frequency of minimum 20—22 Hz for the orbicularis oculi and at 10—12 Hz for the orbicularis oris Apparent fusion occurs at 100 Hz for the orbicularis oculi and at 60 Hz for the orbicularis oris The rate of tension development increases for both muscles with stimulus frequencies up to 200 Hz Average tetanus/twitch ratios are 4 5 for the orbicularis oris and 7 0 for the orbicularis oculi

In the cat polysynaptic reflexes can be elicited in the orbicularis oculi by tapping the skin around the eye and on the nose and similar reflexes are evoked in the auricular muscles by an innocuous mechanical stimulation of the pinna It is obvious that the reflex contractions of the orbicularis oculi and the auricular muscles serve protective functions Such reflexes cannot be evoked in the orbicularis oris muscle by mechanical stimulation of the face (Lindquist and Mårtensson 1970) Instead this muscle serves more sustained motor activity Considering the different functions it may be reasonable to assume that the orbicularis oculi and the orbicularis oris also have different contraction properties This matter is investigated in this and in a following paper (Edström and Lindquist 1973)

Methods

25 tracheotomized cats anesthetized i p with Nembutal® 40 mg/kg b wt were used Blink reflex contractions were recorded also in cats under chloralose anesthesia (50—[illegible]0 mg/kg b wt) *Recording* of contractions from individual facial muscles is very difficult since they lack tendons are often interwoven and sometimes insert into the skin Dissection is thus liable to injure the muscle fibers Most recordings have therefore been made with the skin intact Con



Fig 1 Isometric twitch contractions of the orbicularis oculi (*A*) the depressor conchae (*B*) and the orbicularis oris (*C*) in response to supramaximal stimulation of their respective motor nerve Time bar 20 ms

traction curves from the orbicularis oris were obtained by a string or a metal hook attached to the angle of the mouth and the transducer In a similar way the contractions from the orbicularis oculi were recorded from the upper or lower lid The muscles did probably not pull on the recording instrument with maximal force but this should have no bearing on the interpretation of the results In the majority of the experiments the muscles were activated indirectly by stimulation of their individual motor nerves In this way it is reasonably safe to conclude that the stimulation activated a single muscle whereas direct muscle stimulation increased the possibility of coactivation of other facial muscles The peripheral end of severed nerves was stimulated with bipolar chlorided silver wire electrodes Stimulus pulses of 0 1 ms duration were delivered by a Grass S4 stimulator When needed reexcitation of motor nerve endings was prevented by giving double closely spaced stimulus pulses (*cf* Brown and Matthews 1960)

Near isometric muscle contractions at optimal muscle length were recorded with a Grass FT 03 transducer (self resonance frequency 100—200 Hz) or with a specially constructed barium titanate transducer (selfresonance frequency 2000 Hz) The former was used when the lowest frequency of nerve stimulation giving maximal contractile tension was sought This frequency is referred to as the apparent fusion frequency (*cf* Buller and Lewis 1965) The high sensitivity barium titanate transducer was used to find the frequency of nerve stimulation giving fusion of muscle contractions even when recorded at high amplification This frequency is referred to as the "absolute fusion frequency" The reciprocal of the absolute fusion frequency should be a fair estimate of the duration of the maximal intensity of the active state in a single twitch (*cf* Martensson and Skoglund 1964 This value may also be estimated by the method of MacPherson and Wilkie (1954) and by the method of Gabel, Carson and Vance (1968) By the former method the tension curves resulting from one and two stimuli are compared and the shortest mechanical separation time observed indicates the onset of decay of the active state "plateau The latter method is based on the opinion that the time from the beginning of the twitch to peak tension acceleration coincides with the value for the duration of the active state plateau The tension acceleration is obtained by electronic double differentiation of the twitch tension curve This was accomplished by passing the signal from the transducer through a time constant of 100 μs The transducer was connected to a cathode follower and a Grass P6 dc amplifier The recorded signals were displayed on a Tektronix 502 dual beam oscilloscope

Muscle action potentials were recorded with gross uninsulated steel needles or with bipolar DISA concentric needle electrodes and displayed on the oscilloscope

The temperature in the face was controlled by an infrared heating lamp and kept within 34—39 C which gave consistent values of the contraction times

Results

Twitch contraction characteristic Isometric twitches at optimal muscle length of the orbicularis oculi (*A*) the depressor conchae (*B*) and the orbicularis oris (*C*) in response to supramaximal stimulation of their respective motor nerve are shown in Fig 1 The contraction time is shortest for the orbicularis oculi (8 5 ms) and longest for the orbicularis oris (33 ms) whereas an intermediate value is typical of the depressor conchae (24 ms) Table I summarizes the experimental findings as regard the contraction times of the three muscles Compound action potentials recorded on supramaximal motor nerve stimulation in the muscle with the longest contraction time (orbicularis oris) were of longer duration than those recorded in the muscle with the shortest contraction time (orbicularis oculi) The range of potential

TABLE I Contraction times of facial muscles

Muscle	Range of contraction times ms	Average contraction time ms	Number of experiments
Orbicularis oculi	7.5—10.0	8.5	6
Orbicularis oris	27—35	33	8
Depressor conchae	21—28	24	3

tions for the former muscle was 6.1—9.4 ms and the average duration 8.5 ms, whereas for the latter muscle the range was 2.4—3.0 ms and the average 2.8 ms.

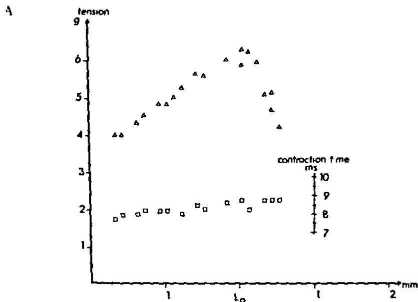
Variations in muscle length have very small effects on the twitch contraction times of the orbicularis oris and oculi. This is borne out by Fig. 2 showing the contraction times of a maximal twitch (squares) of the orbicularis oculi (*A*) and the orbicularis oris (*B*) at lengths shorter and longer than the optimal length (L₀).

The amplitudes of the twitches in relation to the muscle length are also plotted in the same diagrams (triangles). In *A* it can be seen that maximal contraction amplitude is obtained in the orbicularis oculi only if the muscle length is within a few tenths of a mm from the optimal length. On the other hand, virtually maximal contractions in the orbicularis oris are obtained when the muscle length is within a mm from the optimal length. It is obvious that the twitch amplitude of the orbicularis oculi is much more dependent on the muscle length than that of the orbicularis oris and this difference has to be taken into account when comparing the contraction properties of the two muscles. The changes in passive tension (circles in Fig. 2) produced in the two muscles by length variations are comparable.

Homogeneity of the motor unit populations. There are usually differences in contraction times between motor units supplied by nerve fibers of different diameters (see *e.g.* Wuerker, McPhedran and Henneman 1965). Since thresholds for electrical stimulation are related to fiber size it is sometimes possible to distinguish motor units with different contraction times by studying records from muscles obtained when stimuli of different intensities are applied to their motor nerves (*cf.* *e.g.* Andersen and Sears 1964). An experiment with this technique is illustrated in Fig. 3 *A* and *B* which shows several superimposed twitch contractions of the orbicularis oculi and oris respectively obtained at different strengths of stimulation of their nerves. Contractions obtained by stimulation of motor fibers with low electrical thresholds have a time course similar to those obtained by activation of high threshold fibers. This observation indicates that there are no major differences between the contraction times of the individual motor units in the orbicularis oculi and those in the orbicularis oris.

Blink reflex contraction time. Tapping on the cat's facial skin elicits two separate reflex discharges in the orbicularis oculi (Lindquist and Mårtensson 1970), but it is not known whether the same types of motor units discharge in the early and the late component. Knowing the capacity of the orbicularis oculi for quick tension

A



B

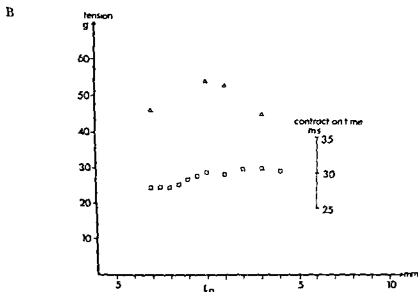


Fig 2 The contraction time (squares) active isometric twitch tension (triangles) and passive tension (circles) of the orbicularis oculi (*A*) and the orbicularis oris (*B*) plotted against muscle length L_0 marks the length giving the largest twitch amplitude



Fig 3 Superimposed recordings of twitch contractions elicited in the orbicularis oculi (*A*) and orbicularis oris (*B*) by stimulation of their motor nerves at various stimulus strengths Time bars 10 ms

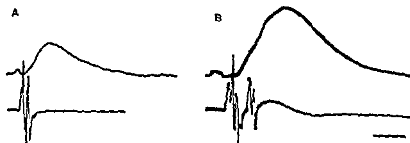


Fig 4 Reflex action potentials (lower beams) and isometric contractions (upper beams elicited in the orbicularis oculi by tap stimuli applied to the ipsilateral medial angle of the eye In *A* only a short latency reflex is produced in *B* also a reflex of longer latency Time bar 15 ms

development it was of interest to see how this is utilized in the blink reflex In several experiments recordings were therefore made of the EMG and the contractile response of the orbicularis oculi to periorbital tap stimulation (*cf* Lindquist and Mårtensson 1970) Fig 4 is from a typical experiment The tap stimulation applied in 4 gives a hort latency reflex action potential (lower beam) The contraction resulting from this discharge (upper beam) reaches a peak in around 15 ms It is evident from this value that the short contraction time of the orbicularis oculi is taken advantage of for rapid protection of the eye The tension produced by a reflex contraction as the one in 4 was often 2—3 times larger than a single twitch elicited by supramaximal stimulation of the orbicularis oculi muscle nerve which implies that some motor units discharge repetitively in the early reflex response

Fig 4 *B* illustrates a situation when in addition to an early response also a late reflex discharge results The second reflex discharge produces a hump in the record and a prolongation of the contractile response The time to peak of the reflex contraction is now 30 ms Since the interval between the two reflex discharges is around 10 ms also the motor units taking part in the longer latency reflex discharge must have short contraction time This is in good agreement with the results presented above showing that the orbicularis oculi is homogeneous as regards contraction times of its motor units

Response to repetitive stimulation Stimulus frequencies giving summation and fusion of contractions vary among muscles in relation to their respective contraction time (Cooper and Eccles 1930 It was therefore considered pertinent to study the responses of the orbicularis oculi and oris to repetitive stimulation of different frequencies

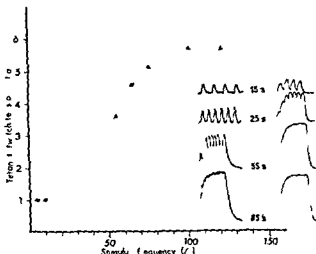


Fig 5 The relation between stimulus frequency and tension developed in the orbi ularis oculi (triangles) and the orbi cularis oris (circles) Inset de scribed in text

The inset of Fig 5 shows muscle contractions elicited in the orbicularis oculi (left hand column) and the orbicularis oris (right hand column) by stimulation of their respective motor nerves at 15 25 55 and 85 stimuli per s in a typical experiment At 15 Hz there is a clearly visible summation of contractions in the orbicularis oris whereas separate twitches are recorded in the orbicularis oculi A small summation is seen in the orbicularis oculi at 25 Hz and the summation in the orbicularis oris is now considerable At 55 Hz two thirds of the maximal tetanic ten ion is produced in the orbicularis oculi and close to maximal tension is attained in the orbicularis oris At 85 Hz finally there is apparent fusion (*cf* Methods) of orbicularis oris contractions but still clearly visible oscillations in the tension output of the orbicularis oculi In Fig 5 isometric tension for the two muscles is plotted against frequency of nerve stimulation It appears that in the frequency range 15—40 Hz the orbicularis oris (circles) re ponds almost linearly and maximally to changes in stimulus frequency For the orbicularis oculi (triangles) this range is larger and shifted to higher fre quencies i 35—80 Hz The findings indicate the possibility of a more precise nervous control of orbicularis oculi contractions

Tetanus/twitch ratios were e timated in six cats and the values in Fig 5 were typical The range of this ratio for the orbicularis oris was found to be 4—5 with an average of 4 5 For the orbicularis oculi the range was 5 5—8 5 and the average tetanus/twitch ratio 7 0

Apparent fusion (*cf* Methods) of orbicularis oculi muscle contractions occurs at around 100 Hz In man discharge frequencies up to 180 Hz have been recorded in single units from this muscle and it has been suggested that impulse frequencies higher than the fusion frequency give an increased rate of tension development (Gordon 1951) Fig 6 illustrates an experiment in which 100 150 and 200 stimuli per second were given to the orbicularis oculi muscle nerve There is a definite



Fig 6 Superimposed recordings of the rising phase of contractions in the orbicularis oculi elicited on repetitive stimulation at 100 150 and 200 Hz

not dramatic increase in the rate of tension development with the higher frequencies of stimulation The maximal rate of tension development should be obtained when the interval between successive stimuli is short enough to keep the contractile elements at their maximal intensity of the active state The duration of the latter may be estimated either by the double stimulation method of MacPherson and Wilkie (1954) or by double derivation of the twitch tension curve (Gabel Carlson and Vance 1968) and should be equal to the reciprocal of the highest value of the absolute fusion frequency (*cf* Methods) recorded with a high sensitivity transducer (Mårtensson and Skoglund 1964) (For technical reasons the double derivation method could only be used in the case of the orbicularis oris) These methods gave values of the duration of the maximal active state intensity in the range of 3 5—5 ms for the orbicularis oculi as well as for the orbicularis oris when the twitch contractions were elicited by supramaximal nerve stimuli The rate of tension development in these muscles may therefore be expected to increase with stimulus frequencies up to 200—300 Hz

The absolute refractory period of the nerve motor endplate muscle membrane system may however limit the frequency response of the muscle It was therefore determined with double nerve stimulation and EMG recording and found to be just below 1 ms for the orbicularis oculi and between 1 2 and 1 5 ms for the orbicularis oris These values are comparable to those obtained for the flexor hallucis longus (1 03 ms) and the soleus (1 61 ms) (Buller and Lewis 1965) The absolute frequency limit for activation of the contractile elements should therefore exceed 300 Hz if it was set by the absolute refractory period of the nerve motor endplate muscle membrane system alone

Following repetitive stimulation twitch contractions of striated muscles are usually potentiated The degree of this post tetanic potentiation (PTP) is higher for fast twitch than for slow twitch muscles (Close 1972) The question was raised whether there are differences also in this respect between the orbicularis oculi and the orbicularis oris muscles PTP was studied following conditioning stimuli of many different frequencies It was found that in both muscles the maximal twitch potentiation is produced by conditioning stimuli of 200—300 Hz and that the PTP is twice as large in the orbicularis oculi at all frequencies

Discussion

The contraction time recorded for the orbicularis oculi is in the same range as the contraction time of the thyroarytenoid (9—13 ms according to Martensson and Skoglund 1964) and of certain extra-ocular muscles (7 5—10 ms for the internal rectus according to Cooper and Eccles 1930) The orbicularis oris is comparable to the cricothyroid (30—35 ms according to Martensson and Skoglund 1964) and to some of the fast extremity muscles (40 ms for the gastrocnemius according to Cooper and Eccles 1930) The contraction time of a muscle is given by the intrinsic speed of shortening of the contractile material the duration of the active state and by the properties of the series-elastic elements (Close 1965) The results obtained in this series of experiments may justify a discussion about the factors responsible for the difference in contraction time between the orbicularis oculi and oris The estimated duration of the maximal intensity of the active state was the same in the two muscles According to Hill (1949) the falling phase of the active state passes through the peak of the twitch contraction Since the maximum tension recorded in tetanic contraction corresponds to the maximal intensity of the active state (*cf* Goffart and Ritchie 1952) it is possible to estimate the rate of decay of the active state if the tetanus/twitch ratio and the contraction time are known (*cf* Martensson and Skoglund 1964) The tetanus/twitch ratio was lower for the orbicularis oris than for the orbicularis oculi and the contraction time longer the rate of fall of the active state in the orbicularis oris is thus slower than in the orbicularis oculi and hence the total duration of the active state is longer in the orbicularis oris This difference may well be one factor determining the difference in contraction time between the two muscles

The cause of the longer duration of the compound action potential recorded in the orbicularis oris has not been specifically investigated Factors such as the propagation velocity of the muscle fibers and the distance over which the potential is propagated may be involved (*cf* *e g* Buchthal Guld and Rosenfalck 1955) A longer duration of the action potential may imply a slower activation of the contractile elements of the muscle It does not however seem likely that this is responsible for the longer contraction time of the orbicularis oris since the compound action potential is prolonged both in the orbicularis oculi and oris during long lasting repetitive stimulation without any significant alteration in the contraction times of the muscles (*cf* Edstrom and Lindquist 1973)

The experimental results indicate that the motor unit population in the orbicularis oculi as well as in the orbicularis oris is homogeneous as regards contraction times of the motor units The agrees well with the fact that a large majority of muscle fibers within the orbiscularis oculi and within the orbicularis oris have a similar activity of myofibrillar ATPase (*cf* Edstrom and Lindquist 1973 which determines the speed of contraction

In his experiments on man Gordon (1951) recorded impulse of frequencies up to 180 Hz in single motor units of the orbicularis oculi during blinking He suggested that these very high discharge frequencies served the purpose of increasin

peed of tension development. The experiments described above show that stimulus frequencies above those necessary for producing apparent fusion of muscular contractions do increase this peed Similar observations have been made also for extremity muscles of the cat (Buller and Lewis 1965) However when comparing the time to peak of the blink reflex in Fig 4 *A* and the contraction time of the maximal isometric twitch of the orbicularis oculi illustrated in Fig 3 *A* no marked difference is apparent in the rate of tension development It is therefore unlikely that high frequency nerve impulses to the orbicularis oculi muscle of the cat serve the purpose of increasing the speed of eye closure in the blink reflex.

References

ANDERSEN P and T A SEARS The mechanical properties and innervation of fast and slow motor units in the intercostal muscles of the cat *J Physiol* (Lond) 1964 *173* 114—129

BROWN M C and P B C MATTHEWS The effect on a muscle twitch of the back response of its motor nerve fibres *J Physiol* (Lond.) 1960 *150* 332—346

BUCHTHAL F C GULD and P ROSENFALCK Innervation zone and propagation velocity in human muscle *Acta physiol scand* 1955 *35* 174—190

BULLER A. J and D M. LEWIS The rate of tension development in isometric tetanic contractions of mammalian fast and slow skeletal muscle *J Physiol* (Lond) 1965 *176* 337—354

CLOSE R. The relation between intrinsic speed of shortening and duration of the active state of muscl *J Physiol* (Lond) 1965 *180* 542—559

CLOSE R Dynamic properties of mammalian skeletal muscles *Physiol Rev* 1972 *52* 129—197

COOPER S and J C ECCLES The isometric responses of mammalian muscles *J Physiol* (Lond 1930 *69* 377—385

EDSTRÖM L and CHR. LINDQUIST Histochemical fiber composition of some facial muscles in the cat in relation to their contraction properties *Acta physiol. scand* 1973 *89* 491—503

GABEL L. P C CARSON and E VANCE Active state of muscle and the second and third derivatives of twitch tension. *Amer J Physiol* 1952 *214* 1025—1030

GOFFART M and J M. RITCHIE The effect of adrenaline on the contraction of mammalian skeletal muscle *J Physiol* Lond 1952 *116* 357—371

GORDON C Observations upon the movements of the eyelids *Brit J Ophthal* 1951 *35* 334—351

HILL A V The abrupt transition from rest to activity in muscle *Proc roy Soc B* 1949 *136* 3[illegible]—420

LINDQUIST CHR and A MÅRTENSSON Mechanisms involved in the cats blink reflex *Acta physiol scand* 1970 *80* 149—159

MACPHERSON L and D P WILKIE The duration of the active state in a muscle twitch *J Physiol* Lond 1954 *124* 29 — 39

MÅRTENSSON A and C R SKOGLUND Contraction properties of intrinsic laryngeal muscle *Acta physiol scand* 1964 *60* 318—336

WUERKER R B A M MCPHEDRAN and E. HENNEMAN Properties of motor units in a heterogeneous pale muscle (m gastrocnemius) of the cat *J Neurophysiol* 1965 *2*[illegible] 85—[illegible]

Acta physiol scand 1973 89 491—503

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Histochemical Fiber Composition of Some Facial Muscles in the Cat in Relation to their Contraction Properties

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Abstract

EDSTRÖM L and CHR LINDQUIST *Histochemical fiber composition of some facial muscles in the cat in relation to their contraction properties* Acta physiol scand 1973 89 491—503

A histochemical analysis has been performed of the activity of myofibrillar ATPase succinic dehydrogenase NADH₂ tetrazolium reductase and phosphorylase and of the content of glycogen and fat in the orbicularis oculi orbicularis oris and the depressor conchae muscles in the cat All three muscles contain fibers with a low activity (type I) and such with a high activity (type II) of myofibrillar ATPase The type II fibers predominate. In general the type II fibers of the orbicularis oris have a higher activity of oxidative enzymes than those of the other two muscles Differences in contraction times in relation to fiber composition are discussed Stimulation of the orbicularis oculi at 5 Hz causes a contractile fatigue of ?2—48 in a 10 min period, which is related to a depletion of glycogen in muscle fibers with a low activity of succinic dehydrogenase The orbicularis oris fatigues very little (2—21 %) during such a period and no significant glycogen depletion is seen This is explained by a higher activity of oxidative enzymes in this muscle

Some facial muscles as for example the orbicularis oculi and the depressor conchae take part in protective reflexes whereas others like the orbicularis oris are more engaged in sustained motor activity In a recent paper (Lindquist 1973) it was shown that these differences between the orbicularis oculi and oris are accompanied by different contraction propertie Thus the orbicularis oculi is a muscle with a very short contraction time (8 5 ms) a high tetanus/twitch ratio (7 and a high apparent fusion frequency (100 Hz) The orbicularis oris on the other hand has a longer contraction time 33 ms) a lower tetanus twitch ratio (4 5 and a low fusion frequency (60 Hz

By histochemical method a correlation can be made between the contraction properties of muscles and their substrate content and enzyme activity (for review see *e g* Close 1972) Thus it has been shown in the rat that the anterior tibial muscle is much more susceptible to fatigue than the soleus and that the muscle fatigue runs parallel with a depletion of glycogen in muscle fibers with a low

TABLE I Frequency and size of type I and type II fibers in the facial muscles studied

Muscle	Frequency (%)		Mean fiber cross-section area (100 μm^2)		% of cross section	
	type I	type II	type I	type II	type I	type II
Orbicularis oculi	10	90	2.5	10.7	3	97
Orbicularis oris	15	85	3.5	9.1	6	94
Depressor conchae	30	70	7.0	7.6	28	72

activity of oxidative enzymes (Kugelberg and Edström 1968) In the present paper a comparison is made of the fiber composition of the orbicularis oculi and oris as well as of the depressor conchae muscles The fatiguability of the former muscles is correlated with the depletion of glycogen in some of their muscle fibers during prolonged low frequency stimulation The analysis of the fiber composition and the fatigue studies show that the orbicularis oris is better suited for tonic functions than the orbicularis oculi. The relation between previously obtained values of contraction times of the orbicularis oculi the orbicularis oris and the depressor conchae and the fiber composition of the three muscles is discussed.

Methods

Experiments were performed on 10 tracheotomized cats (2.5—3.5 kg) anesthetized by i.p. injections of 40 mg Nembutal® per kg b.wt. The surgical procedures and the recording and stimulation equipment used for the physiological studies have been described in detail elsewhere (Lindquist 19[illegible]3). A Grass FT 03 force-displacement transducer was used The muscle contractions were displayed on an oscilloscope and on a Grass polygraph model 5

Fatigue experiments Before onset of the fatigue experiments the length of the muscles was adjusted to give maximal contractions. Care was taken not to overstretch the muscles or in any other way interfere with the blood supply Interference with the blood supply was minimized also by limiting the study mainly to effects of stimulation frequencies producing twitch contractions (cf Lindquist 19[illegible]3) thus preserving the blood pumping action of the muscles. The muscle temperature was kept within the physiological range by infrared heating lamps The development of muscle fatigue was studied during 10 min periods of stimulation at 5 [illegible]0 Hz since in our experience there was a very small further decline in the muscle contractions even with much longer periods of stimulation

Histochemical procedure The muscle biopsy material was immediately frozen in liquid nitrogen Sections 15 μm thick were cut in a cryostat *Staining methods* NADH₂ [illegible] reductase Scarpelli Hess and Pearse 1958 succinic dehydrogenase Nachlas *et al* 195[illegible] lipids Carleton and Drury 1957 myofibrillar adenosine triphosphatase ATPase Padykula and Herman 1955 phosphorylase (Takeuchi and Kuriaki 1955 modified by Frank and Palkama 1961) and glycogen Mowry and Millican 1952 Some slight modifications were made see Nyström 1968 For further details see also Pearse 1960

By photographic registration and comparison of consecutive cross sections stained by the different methods used the staining properties of different muscle fibers could be studied

The size of the muscle fibers was determined by area estimation by the method of Edström and Torlegård 196[illegible]

Results

General histochemical characteristics of muscle fibers

Myofibrillar ATPase Two distinct fiber types are easily distinguished in all three muscles investigated The fibers with a low activity of myofibrillar ATPase appearing light in the sections of the orbicularis oris (Fig 1 A and 2 A) the orbicularis

A C

B D

Fig 3 Serial cross sections from orbicularis oculi muscle *A* myofibrillar ATPase *B* succinic dehydrogenase *C* phosphorylase *D* glycogen (PAS) Arrow indicates position of a type I fiber o position of 2 type II fibers × 175

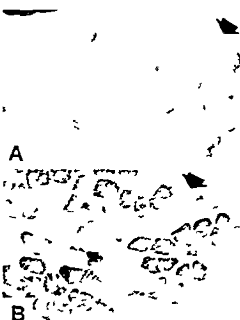


Fig 4 Serial cross sections from depressor oncha muscle *A* m fibrillar ATPa *B* succi i det drogenase "x in licates p tion f a type I fiber arrow points to a type II fiber × 1 0

TABLE II Histochemical staining reactions of facial muscle fibers

	type I		type II		
myofibrillar ATPase	low	low	high	high	high
phosphorylase	low	low	high	high	high
glycogen	low	low	high	high	high
succinic dehydrogenase	high	intermediate	high	intermediate	low
NADH tetrazolium reductase	high	intermediate	high	intermediate	low
fat	high	intermediate	high	intermediate	low

oculi (Fig 3 A) and the depressor conchae (Fig 4 A) are called type I fibers according to the nomenclature of Fenichel and Engel (1963) The fibers with a high activity of the enzyme appearing dark in the same sections are called type II according to the same nomenclature The size and frequency of type I and II fibers in the individual muscles are summarized in Table I The proportion of type I and type II fibers in the individual muscles in relation to their contraction times is considered in the Discussion

Oxidati e enzymes and fat In Fig 1 a type I fiber from the orbicularis oris—lightly stained for myofibrillar ATPase in A—is indicated by an arrow in consecutive sections stained for succinic dehydrogenase (*B*) NADH tetrazolium reductase (*C*) and fat (*D*) The fiber is well supplied with the two oxidative enzymes and fat Other type I fibers have an intermediate degree of staining for the oxidative enzymes and fat In the same figure two type II fibers— darkly stained for myofibrillar ATPase in A— are indicated by an o The upper left of these two fibers has a high and the lower right fiber an intermediate activity of the two oxidative enzymes and content of fat (*B* *C* and *D*) Type II fibers with a low activity of the two oxidative enzymes and low fat content are also found in all three muscles (*cf* Fig 2—4)

Although fibers with low intermediate or high activity of oxidative enzymes and fat are all present it is characteristic that in the individual fiber the color reactions for the two oxidative enzymes and fat run parallel This is in agreement with the staining pattern of extremity muscles in mammals Fibers with an inverse relationship between succinic dehydrogenase and NADH tetrazolium reductase activity are found in ome of the intrinsic laryngeal muscles of the cat (Edstrom Lindquist and Mårtensson 1973) but could not to any significant extent be identified in the thr… facial muscles

Phosphorylase and glycogen In Fig 3 the staining reactions for myofibrill… ATPase (A) succinic dehydrogenase (*B*) phosphorylase (*C*) and glycogen (*D*) compared in consecutive cross sections from the orbicularis oculi muscle An … marks the position of a type I fiber which reacts weakly for myofibrill… phosphoryla e and glycogen but gives an intermediate reaction fo… hydrogenase An o marks the position of two type II fibers in … They exhibit a high activity of myofibrillar ATPase phosphoryl… glycogen and a low activity of succinic dehydrogenase

The phosphorylase activity and glycogen content is thus largely parallel with the activity of myofibrillar ATPase i e low in type I and high in type II fibers This conforms with the findings in e g the gastrocnemius of the cat (Nystrom 1968)

The various fiber types found in the three facial muscles investigated are summarized in Table II It appears that there are two kinds of type I fibers and three kinds of type II fibers It must however be emphasized that the expression intermediate for oxidative enzymes and fat corresponds to a spectrum of levels between high and low

Fiber composition of individual muscles

Orbicularis oris The fiber composition varies in different parts of the muscle

In Fig 1 *A* is shown a cross section from the predominant peripheral part of the muscle stained for myofibrillar ATPase It is obvious that most fibers are darkly stained and thus of type II (about 90 %)

Fig 2 *A* shows the smaller central part of the muscle stained for myofibrillar ATPase This part exhibits a higher frequency of type I fibers (about 30 %)

The estimated mean area of individual type I fibers is 350 µm and that of the type II fibers 910 µm for the muscle as a whole The type I fibers tend to be larger in the peripheral part of the muscle

Fig 1 *B* and 2 *B* represent sections stained for succinic dehydrogenase and demonstrate another difference between the two parts of the muscle In the peripheral part (Fig 1 *B*) most of the fibers have a high or intermediate staining reaction for succinic dehydrogenase In the central part of the muscle (Fig 2 *B*) there are large sized type II fibers reacting weakly for succinic dehydrogenase n [illegible] ve of such fibers is the left one of the two fibers indicated by an o Fig 2 *A* and *B* These large fibers with low staining reactions for oxidative enzymes have the staining characteristics of the predominating type II fibers in the orbicularis oculi but differ from them in that they keep up their glycogen stores on stimulation at 5 Hz (*cf* below)

The orbicularis oris as a whole is composed mainly of fibers with an intermediate staining reaction for oxidative enzymes which should explain the higher fatigue resistance in comparison with the orbicularis oculi (*cf* below)

Orbicularis oculi Fig 3 *A* shows a cross section from the muscle stained for myofibrillar ATPase In this muscle there is an even more pronounced difference in size between type I and type II fibers Because of their small size (250 μm^2) the type I fibers constitute only about 3 % of the cross sectional muscle area The type II fibers are of about the same size as the corresponding fibers in the orbicularis oris (mean area 1070 µm) Among the type II fibers—making up 97 % of the muscle cross section—there are also considerable differences in size between individual fibers and these proved to be related to differences in reactivities for oxidative enzymes e g succinic dehydrogenase Thus fibers with a high activity of succinic dehydrogenase tend to be small and fibers with a low activity of this enzyme



Fig 5 Twitch contractions (upper records) and action potentials (lower records) from orbicularis oculi (*A*) and orbicularis oris (*B*) in response to supramaximal stimulation of their respective motor nerve 10 min between arrows

to be large (*cf* Fig 3 *B* and 7 *A* *C*) This relation between staining reaction for oxidative enzymes and fiber size is common in extremity muscles of mammals (*cf* Ogata 1958 Dubowitz and Pearse 1960) but is not universal (Edstrom and Nystrom 1969 Ringqvist 1971)

The large type II fibers with low reactivity for oxidative enzymes are the predominant fibers in this muscle covering about 70 % of its cross section It is the type of fiber that should be easily fatigued during long lasting low frequency stimulation as a result of glycogen depletion (*cf* below)

Depressor conchae In this muscle the type I fibers are larger and the type II fibers smaller than in the other two muscles studied (700 μm and 760 μm respectively) Moreover the frequency of type I fibers is comparatively high (30 %)

Fig 4 shows a serial section from the depressor conchae muscle stained for myofibrillar ATPase (*A*) and succinic dehydrogenase (*B*) It is obvious that the type II fibers (darkly stained in *A*) exhibit a mixture of fibers with different reactivity for succinic dehydrogenase (*B*) The relation between stainability and size is less pronounced than in *e g* the orbicularis oculi and the central part of the orbicularis oris where large sized fibers with low staining reaction for succinic dehydrogenase are prominent (*cf* Fig 2 *B* and 3 *B*)

Structures resembling muscle spindles were not encountered in any of the muscles investigated

Fatigue studies

Fig 5 is a typical illustration of the development of fatigue in the orbicularis oculi (*A*) and the orbicularis oris (*B*) when a stimulus of 5 Hz is applied to the respective muscle nerve (The muscles were studied one by one but the recordings are shown together to facilitate comparisons) The upper record in *A* and *B* show the



Fig 6 Compound action potentials recorded from orbicularis oculi at start (A) 3 min (B) and 6 min (C) after start of supramaximal stimulation of the muscle nerve at 10 Hz

contractile responses and the lower records the compound muscle action potentials. Both muscles were fresh when the experiment started with 1 Hz stimulation The recording in Fig 5 starts when twitch contractions of stable amplitude are obtained At the point indicated by the arrow pointing upward the stimulus frequency is suddenly increased to 5 Hz The action potentials *are not affected by this change in* either muscle but a staircase effect is noted in the orbicularis oculi with an increase of the twitch amplitude by approximately 20 % in 20 s In the same period of time *there is only a 1 % increase in the orbicularis* oris twitch tension During the 10 min stimulation period there is then a progressive decline of twitch tension in both muscles which is however much more pronounced in the orbicularis oculi In this muscle 33 % of the maximal twitch tension at 5 Hz has been lost after 10 min just before the stimulus frequency is switched back to 1 Hz The corresponding value of the orbicularis oris is only 12 % The action potential recordings are unchanged throughout the stimulation period indicating that the decline in tension is due to contractile fatigue rather than failure of neuromuscular propagation At the arrow pointing downwards in Fig 5 the stimulus frequency is suddenly brought back to 1 Hz Twitch contractions with post tetanic potentiation are then recorded in both muscles This potentiation gradually subsides and a twitch amplitude is finally reached which for the *orbicularis oris is 21 % lower than that at 1 Hz* before the 5 Hz stimulation period for the orbicularis oculi the depression of the twitch tension is however more pronounced and amounts to 48 % Since there is a considerable *difference in loss* of contractile tension also when comparing the two muscles at 1 Hz following the fatigue period and after the disappearance of post tetanic potentiation phenomena it must be concluded that there is a real dissimilarity in fatiguability and not only a difference due to the pronounced but temporary staircase effect in the orbicularis oculi

In all experiments the orbicularis oris was much more resistant to fatigue than the orbicularis oculi but the degree of fatigue varied considerably for the individual muscles from experiment to experiment The experimental findings at a stimulation frequency of 5 Hz are summarized in Table III

TABLE III Twitch tension decline on stimulation at 5 Hz for 10 min

	Recorded at 5 Hz		Recorded at 1 Hz (see text)	
	average	range	average	range
Orbicularis oculi	43	27–57	35	2?–48
Orbicularis oris	11	0–22	13 °	2–21



Fig 7 Comparison between orbicularis oculi muscles unstimulated (*A* and *B*) and stimulated at 5 Hz for 10 min (*C* and *D*) stained for succinic dehydrogenase (*A* and *C*) and glycogen (*B* and *D*) Similar arrows point to corresponding fibers The stimulated muscles exhibit a selective depletion of glycogen from fibers with low reactivity for succinic dehydrogenase Two arrows indicate groups of such fibers in *C* and *D* × 180

At stimulus frequencies of 10 and 20 Hz there is in principle still the same difference in fatiguability between the two muscles but there is also a considerable fatigue of the orbicularis oris at these frequencies (20—45 % lower twitch tension at 1 Hz after a 10 min stimulation period) In these experiments changes also occur in the compound muscle action potentials Fig 6 shows such a potential in the orbicularis oculi just following the start of stimulation at 10 Hz (*A*) after 3 min (*B*) and after 6 min (*C*) There is an obvious decline of the potential and the duration is increased Calculations of the area covered by the action potential show however that it is the same throughout the period of stimulation This indicates that the number of activated muscle fibers is not altered The functional significance of the action potential changes is discussed below

After termination of the fatigue experiments the muscles were removed and freshly frozen for histochemical examination (*cf* Methods) Fig 7 shows serial

sections from orbicularis oculi muscles stained for succinic dehydrogenase (A and C) and for glycogen (B and D). A and B are sections from an unstimulated control and C and D sections from a muscle stimulated at 5 Hz for 10 min. Most muscle fibers in the control sections are well supplied with glycogen as demonstrated in B. In the muscle stimulated at 5 Hz there is an almost complete depletion of glycogen in all fibers (D) with low staining reaction for succinic dehydrogenase (cf. C). Groups of such fibers are indicated by two arrows to facilitate comparison between C and D. Fibers with high or intermediate reactivity for succinic dehydrogenase are unaffected.

This selective effect of low frequency stimulation on fibers with a low activity of succinic dehydrogenase has earlier been demonstrated by Kugelberg and Edström (1968) on the anterior tibial muscle of the rat. The stainability for succinic dehydrogenase was not affected by the stimulation (compare Fig. 7, A and C).

In the orbicularis oris muscle no significant changes in the glycogen content could be established after stimulation at 5 Hz. This resistance to low frequency stimulation found in the orbicularis oris is comparable to the findings in the soleus muscle of the rat (Kugelberg and Edström 1968) and in some of the internal laryngeal muscles of the cat (Edström, Lindquist and Mårtensson 1973).

The orbicularis oris muscle contains some fibers with low stainability for succinic dehydrogenase which are not found in the soleus of rat and cat and which should be expected to lose their glycogen after stimulation at 5 Hz. They were however unaffected in the same way as fibers with the same staining characteristics found in some intrinsic laryngeal muscles (Edström, Lindquist and Mårtensson 1973). Thus the fact that a fiber has low reactivity for oxidative enzymes and fat does not always indicate that it can easily mobilize its glycogen during low frequency motor nerve stimulation.

Discussion

The results from the histochemical studies of the muscle activity of myofibrillar ATPase show that 70 % of the fibers in the depressor conchae muscle, 90 % of those in the orbicularis oculi and 85 % of those in the orbicularis oris have a high activity of this enzyme. The biochemically estimated activity of this ATPase in smooth and striated muscle of invertebrates as well as of vertebrates is higher the shorter the contraction time of the muscle (Bárány 1967). Furthermore it has been shown with a histochemical mapping technique (Edström and Kugelberg 1968) that fast twitch motor units in the rat soleus muscle (Kugelberg 1971 and 1973) as well as in the cat gastrocnemius muscle (Burke, Levine and Zajac 1971) are composed of fibers rich in myofibrillar ATPase (type II) and that slow twitch units are made up of fibers with a low activity of this enzyme (type I). The correlation between the relative number of type II fibers and the contraction times for some cat muscles supplied by cranial nerves has recently been established (for the middle ear muscles by Teig and Dahl (1972), for the jaw muscles by Bosley, Cody and Taylor (1972) and for some of the intrinsic laryngeal muscles by Edström, Lindquist and

Martensson (1973) The contraction times for the orbicularis oculi the depressor conchae and the orbicularis oris muscles are 8 5 24 and 33 ms respectively (Lindquist 1973) Considering their pronounced differences in contraction time it may seem remarkable that the percentage of type II fibers (fibers rich in myofibrillar ATPase) does not differ very much in the orbicularis oris and the orbicularis oculi muscles

The *staining intensity for myofibrillar ATPase should reflect the rate by which* myosin may hydrolyze ATP It has been suggested that this rate determines the speed of the intrinsic shortening of a muscle In turn it is mainly differences in the intrinsic speed of shortening that seem to account for dissimilarities in twitch contraction times between striated muscles (Close 1965) However other factors such as the properties of the series elastic elements and the time course of the active state must also be taken into consideration There is a difference in the rate of fall of the active state between the orbicularis oculi and the orbicularis oris and the possibility that this may explain the observed differences in twitch contraction times between the two muscles has previously been discussed (Lindquist 1973) It may well be that the activity of myofibrillar ATPase bears no direct relationship to the time course of the active state

The fatigue studies point to a clear connection between the histochemically demonstrable glycogen remaining in the facial muscles after prolonged low frequency stimulation and the contractile tension Muscle fibers with a low activity of oxidative enzymes of the kind predominant in the orbicularis oculi lose their glycogen more easily than fibers with an intermediate or high activity of oxidative enzymes The latter *type of fiber predominates in the orbicularis oris and this muscle is also more* fatigue resistant than the orbicularis oculi These observations are consistent with those made on extremity muscles of the rat (Kugelberg and Edstrom 1968 Edstrom and Kugelberg 1968) and on the anterior tibial the soleus and some of the intrinsic laryngeal muscles of the cat (Edstrom Lindquist and Martensson 1973) Thus it seems quite clear that exhaustion of the energy stores is a cause of muscular fatigue at low frequencies of activation However results from some recent experiments on frog muscles *in vitro* indicate that the contractile power is drastically lowered due to failure in the coupling between excitation and contraction before the energy stores are completely exhausted (Grabowski Lobsiger and Luttgau 1972)

In the course of stimulation at 10 Hz the action potential amplitudes decreased whereas their durations increased resulting in an unchanged area covered by the action potentials The number of activated muscle fibers should be proportional to the action potential area and it may therefore be assumed that the number of activated muscle fibers was constant throughout the experiment The increase in potential duration may be caused by a delay in activation of certain muscle fibers (*cf* Dahlback Ekstedt and Stalberg 1970) resulting in a more asynchronous activation of the muscle as a whole Such an activation may possibly cause a lower than normal twitch amplitude of a muscle with a contraction time as short as that of the orbicularis oculi A mechanism of this kind may contribute to the more

muscle fatigue observed at 10 Hz as compared to that at 5 Hz although the main part of the difference in fatigue is probably explained by a greater exhaustion of the contractile elements. At still higher frequencies of stimulation it may be expected that the delayed activation of certain muscle fibers turns into a virtual block of the neuromuscular transmission. As early as 1942 del Pozo pointed out that muscular fatigue at low frequencies of stimulation is probably caused by failure of the contractile elements and muscular fatigue at high stimulus frequencies (more than 20—30 Hz) by failing neuromuscular transmission. On maximal voluntary contraction the first interosseus muscle of man fails at first rapidly by neuromuscular fatigue and then more slowly by a successive exhaustion of the contractile elements (Stephens and Taylor 1972).

Together with the results from previous studies of the contraction properties of facial muscles (Lindquist 1973) the present findings regarding enzyme activity and substrate content as well as fatigue properties of the facial muscles show that these muscles do not form a homogeneous group but that there is a clear functional differentiation between them. Thus the orbicularis oculi muscle is well suited for rapid actions required for effective performance in protective reflexes but less well adapted for tonic contractions. On the other hand the orbicularis oris is well suited for tonic functions but less well for rapid actions.

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References

BÁRÁNY M. ATPase activity of myosin correlated with speed of muscle shortening. *J gen Physiol* 196 50 19 218.

BOSLEY M A, F W J COOPER and A TAYLOR. The correlation of histochemistry and speed of contraction in cat jaw muscles. *J Physiol* (Lond) 197 2 4 92 P.

BURKE R E, D N LEVINE and F E ZAJAC III. Mammalian motor units: physiological histochemical correlation in three types in cat gastrocnemius. *Science* 1971 *174* 0 —1 .

CARLETON H M and R A B DRURY. *Histological technique* 3rd ed. Oxford Univ Press, London 1957.

CLOSE R. The relation between intrinsic speed of shortening and duration of the active state of muscle. *J Physiol* (Lond) 1965 *180* 542—554.

CLOSE R. Dynamic properties of mammalian skeletal muscles. *Physiol Rev* 1972 52 129—197.

DAHLBÄCK L O, J EKSTEDT and E STÅLBERG. Ischemic effects on impulse transmission to muscle fibers in man. *Electroenceph clin Neurophysiol* 1970 29 579—591.

DUBOWITZ V and A G E PEARSE. A comparative histochemical study of oxidative enzyme and phosphorylase activity in skeletal muscle. *Histochemie* 1960 2 105—117.

EDSTRÖM L and E KUGELBERG. Histochemical composition, distribution of fibres and fatiguability of single motor units. Anterior tibial muscle of the rat. *J Neurol Neurosurg Psychiat* 1968 *31* 424—433.

EDSTRÖM L and B NYSTRÖM. Histochemical types and sizes of fibres in normal human muscles. *Acta neurol scand* 1969 45 25 —269.

EDSTRÖM L and K TORLEGÅRD. Area estimation of transversely sectioned muscle fibres. *Zeiss Mitt* 1969 69 166—178.

EDSTRÖM L, CHR LINDQUIST and A MARTENSSON. Correlation between functional and histochemical properties of the intrinsic laryngeal muscles in the cat. *International symposium on ventilatory and phonatory control systems* London 1972. Oxford Univ Press. In press 1973.

ERÄNKÖ O and A PALKAMA. Improved localization of phosphorylase by the use of polyvinyl pyrrolidine and high substrate concentration. *J Histochem Cytochem* 1961 9 585.

FENICHEL G M and W K ENGEL. Histochemistry of muscle in infantile spinal muscular atrophy. *Neurology* 1963 13 1059—1066.

GRABOWSKI W, E A LOBSIGER and H CH LUTTGAU The effect of repetitive stimulation at low frequencies upon electrical and mechanical activity of single muscle fibres *Pflugers Arch ges Physiol* 1972 *334* 222—239

KUGELBERG E Distribution histochemical and physiological properties of normal and reinnervated rat motor units In *Abstracts of communications and invited reports at the Fourth International Congress of Electromyography* Brussels 1971 pp 85—86

KUGELBERG E Properties of rat hind limb motor units In *New Development in Electromyography and Clinical Neurophysiology* Ed J E Desmedt Karger Basel 1973 *1* 2—13

KUGELBERG E and L EDSTROM Differential histochemical effects of muscle contractions on phosphorylase and glycogen in various types of fibres relation to fatigue *J Neurol Neurosurg Psychiat* 1968 *31* 415—423

LINDQUIST CHR Contraction properties of cat facial muscles *Acta physiol scand* 1973 *89* 482—490

MOWRY R W and R C MILLIGAN A histochemical study of the distribution and fate of dextran in tissues of the mouse *Amer J Pathol* 1952 *28* 522

NACHLAS M M K C TSOU E DE SOUZA C S CHENG and A M SELIGMAN Cytochemical demonstration of succinic dehydrogenase by the use of a new p nitrophenyl substituted ditetrazole *J Histochem Cytochem* 1957 *5* 420—436

NYSTROM B Histochemistry of developing cat muscle *Acta neurol scand* 1968 *44* 405—439

OGATA T A histochemical study of red and white muscle fibres Part I Activity of the succinoxydase system in muscle fibres *Acta Med Okayama* 1958 *12* 216—227

PADYKULA H A and E HERMAN The specificity of the histochemical method for adenosine triphosphatase *J Histochem Cytochem* 1955 *3* 170—183

PEARSE A G E *Histochemistry theoretical and applied* 2nd ed J and A Churchill Ltd London 1960

DEL POZO E C Transmission fatigue and contraction fatigue *Amer J Physiol* 1942 *135* 763—771

RINGQVIST M Histochemical fiber types and fiber sizes in human masticatory muscles *Scand J Dent Res* 1971 *79* 366—368

SCARPELLI D R HESS and A G E PEARSE The cytochemical localization of oxidative enzymes I *J biophys biochem Cytol* 1958 *4* 747—752

STEPHENS J A and A TAYLOR Fatigue of maintained voluntary muscle contraction in man *J Physiol* (Lond) 1972 *220* 1—18

TAKEUCHI T and H KURIAKI Histochemical detection of phosphorylase in animal tissues *J Histochem Cytochem* 1955 *3* 153—160

TEIG E and H A DAHL Actomyosin ATPase activity of middle ear muscles in the cat *Histochemie* 1972 *29* 1—7

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References

BÁRÁNY, M.: ATPase activity of myosin correlated with speed of muscle shortening. *J. gen. Physiol.* 1967. *50.* 197—218.

BOSLEY, M. A., F. W. J. CODY and A. TAYLOR: The correlation of histochemistry and speed of contraction in cat jaw muscles. *J. Physiol.* (Lond.) 1972. 224. 92 P.

BURKE, R. E., D. N. LEVINE and F. E. ZAJAC III: Mammalian motor units: physiological-histochemical correlation in three types in cat gastrocnemius. *Science* 1971. *174.* 709—712.

CARLETON, H. M. and R. A. B. DRURY: *Histological technique.* 3rd ed. Oxford Univ. Press London 1957.

CLOSE, R.: The relation between intrinsic speed of shortening and duration of the active state of muscle. *J. Physiol.* (Lond.) 1965. *180.* 542—559.

CLOSE, R.: Dynamic properties of mammalian skeletal muscles. *Physiol. Rev.* 1972. 52. 129—197.

DAHLBÄCK, L. O., J. EKSTEDT and E. STÅLBERG: Ischemic effects on impulse transmission to muscle fibers in man. *Electroenc. ph. clin. Neurophysiol.* 1970. *29.* 579—591.

DUBOWITZ, V. and A. G. E. PEARSE: A comparative histochemical study of oxidative enzyme and phosphorylase activity in skeletal muscle. *Histochemie* 1960. 2. 105—117.

EDSTRÖM, L. and E. KUGELBERG: Histochemical composition, distribution of fibres and fatiguability of single motor units. Anterior tibial muscle of the rat. *J. Neurol. Neurosurg. Psychiat.* 1968. *31.* 424—433.

EDSTRÖM, L. and B. NYSTRÖM: Histochemical types and sizes of fibres in normal human muscles. *Acta neurol. scand.* 1969. *45.* 257—269.

EDSTRÖM, L. and K. TORLEGÅRD: Area estimation of transversely sectioned muscle fibres. *Z. wiss. Mikr.* 1969. *69.* 166—178.

EDSTRÖM, L., CHR. LINDQUIST and A. MÅRTENSSON: Correlation between functional and histochemical properties of the intrinsic laryngeal muscles in the cat. *International symposium on ventilatory and phonatory control system.* London 1972. Oxford Univ. Press. In press 1973.

ERÄNKÖ, O. and A. PALKAMA: Improved localization of phosphorylase by the use of polyvinyl pyrrolidine and high substrate concentration. *J. Histochem. Cytochem.* 1961. *9.* 585.

FENICHEL, G. M. and W. K. ENGEL: Histochemistry of muscle in infantile spinal muscular atrophy. *Neurology* 1963. 13. 1059—1066.

preliminary report of this investigation has been published (Körner and Landgren 1969)

Methods

22 cats were used for the experiments The anesthesia was induced by Halothane (Hoechst) vaporized in a Fluotec apparatus by a gas mixture (2 1) of O_2 and N_2O It was maintained with chloralose (0 mg/kg i.v.) Prior to making spinal cord lesions the animal was paralysed with Flaxedil (May and Baker) after which ventilation was artificially maintained with 96 O_2 and 4 % CO_2. Blood pressure body temperature and temperature of the paraffin pools were routinely monitored

The following contralateral nerves were dissected cut, and prepared for electrical stimulation the branch of the musculocutaneous nerve to the elbow joint capsula (Ejt) the posterior knee joint nerve (Kjt) the superficial radial nerve (SR) the deep radial nerve (DR) the sural nerves (Su) the nerves to the gastrocnemius-soleus muscles (G) and to the posterior biceps-semitendinosus muscles (BSt) Ejt was dissected and identified as described by Andersen *et al* (1967) The terms contralateral and ipsilateral are when not otherwise stated used with reference to the investigated cerebral hemisphere

Laminectomies were made in the lumbar (L_4—L_7) thoracic (Th_5—Th_{10}) or cervical (C_1 or C_2—C_4) regions The first somatic areas of both cerebral hemispheres were exposed The pericruciate region of one hemisphere was then removed as described by Landgren and Silfvenius (1969) The medial surface of the other posterior sigmoid gyrus was thus exposed.

Graded electrical stimulation of the afferent nerves was provided by a stimulator having square wave pulse output of 100 µs duration. Single pulses as well as a train of 3 pulses at 600—650 Hz were used Both types of stimuli were applied in turn when testing and recording from each cortical point. The responses evoked by single shocks and by trains were similar though those evoked by the train had larger amplitudes. Response latencies were measured from the artefact of the single pulse or the first pulse of the train to the initial deflection of the evoked potential Stimulus strengths and thresholds of the evoked potentials were expressed in multiples of the strength (T) required to evoke a threshold response in the afferent nerve The afferent volley was monitored from the radial, musculocutaneous and tibial nerves and from the L_6 or L dorsal roots Evoked potentials were recorded from the surface of the cortex by a spring mounted silver ball electrode (ball diameter 0 5 mm) Each area was mapped with points spaced about 1 mm and the recording positions were marked on a photograph of the area using the vascular pattern as landmarks In two experiments the evoked potentials were recorded from the surface with a microelectrode moved in 200 µm steps along antero-posterior and medio-lateral lines across the Kjt projection areas of the dorsal posterior sigmoid gyrus The deeper layers of the cortex were then penetrated with the microelectrode near the points where the evoked potentials showed the largest amplitude (i e at the maximal point of each locus) The caudal bank of the coronal sulcus was also explored with microelectrodes in order to locate the Ejt projections to this region

The cortical evoked potentials the afferent nerve volleys and the stimulus pulses were displayed on a modified Tectronix Model 565 oscilloscope and photographed Signal averaging was sometimes used to improve the signal to noise ratios of the cortical potentials Usually 40 traces were averaged on a Didac Physioscope Mod 1800

The extents of the spinal lesions were verified histologically in serial sections stained with Luxol fast blue (Kluver and Barrera 1953

Results

The components of the afferent volleys in the joint nerves

The compound action potentials produced by electrical stimulation of Ejt or Kjt and recorded from the peripheral nerve truncs generally displayed 3 characteristic components as described by Andersen *et al* (1967) and by Lindstrom and Takata (1972) The first 2 components were evoked as a rule by near threshold stimulus strengths Their development with increasing stimulus strength may be followed in

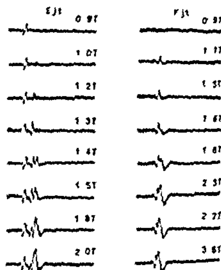


Fig. 1. Records of compound action potentials evoked in the elbow joint nerve (Ejt) and the posterior knee joint nerve (Kjt). Strengths of electrical stimulations of nerves expressed in multiples of threshold intensity (T).

Fig. 1. These two components will be referred to as low threshold in this report. A third, higher threshold component became apparent at strengths around 2T. It was sometimes obscured in triphasic recordings from the intact nerve but was revealed in monophasic records. The stimulus strengths required to evoke the components in several animals are given in Table I. The threshold of component II was generally found between 1.4 and 1.6 T, thus somewhat below the strength necessary to evoke a component I of maximal amplitude. Exceptions were observed in 7 out of 16 Kjt preparations in which the thresholds of component II were higher, between 1.7 and 2.4 T. One Kjt preparation displayed three components in the low threshold range.

Graded electrical stimulation of the joint nerves cannot, of course, be relied upon to selectively stimulate axons from defined receptor populations. However, anatomical studies of the receptor organs and their fibres (Gardner 1944, Skoglund 1956, Freeman and Wyke 1967) and measurements of the fibre conduction velocities and studies of receptor discharge patterns (Andrew 1954, Boyd 1954, Andersson et al. 1967, Burgess and Clark 1969 a, b) strongly suggest that the component I of Ejt and Kjt is due for the most part to fibres from Paciniform corpuscles in the joint capsule and Golgi type endings in the joint ligaments, and that the component II is due largely to the fibres from the Ruffini endings of the joint capsule. Therefore, the subdivision of afferent volleys into components, although somewhat artificial in physiological terms, nonetheless can give information about the central projections of these joint receptors. Furthermore, electrical stimulation provides a means for producing a synchronous input and accurate timing of stimulus and response features that facilitate the analysis. Therefore, the method was adopted in spite of limitations due to probable activation of overlapping populations of joint afferents and also of fibres innervating receptors not yet identified histologically (Burgess and Clark 1969 b).

TABLE I Stimulus strengths required to evoke the components of the compound action potentials in the Ejt and Kjt nerves Stimulus strength is given in multiples of the strength (T) which evokes a threshold response in the nerve trunk

	Ejt				
	Comp I		Comp II		Comp III
	Threshold	Ampl max	Threshold	Ampl max	Threshold
N	17	11	17	14	9
Mean	1 00	1 66	1 33	2 5?	1 98
S D		0 46	0 13	0 65	0 60
Range		1 2—2 5	1 1—1 5	1 8—4 1	1 ?—3 5

N = number of investigated animals
Comp = component
Ampl max = amplitude maximum

The cortical topography of the low threshold Ejt projections

Electrical stimulation of low threshold Ejt afferents evoked initially positive surface potentials in three different loci in the contralateral postsigmoid and coronal gyri 1) in the region of the postcruciate dimple (Pcd) 2) in or near the caudal bank of the coronal sulcus and 3) near the lateral end of the cruciate sulcus (in area 4 ,) These three responding areas will be referred to as the cortical Ejt loci Responses were observed in the Pcd locus in all of 9 brains investigated Coronal loci were found in 5 of 8 animals tested and in 3 of these the coronal loci were traced with microelectrodes and found along the caudal bank of the coronal sulcus near its caudal end Two of the cats had projection fields on the surface of the coronal gyrus near the caudal end of the sulcus Three of the 8 investigated animals showed only high threshold Ejt surface potentials in the coronal gyrus however the coronal sulcus was not investigated in these animals Low threshold Ejt potentials were evoked in area 4 , in 5 of 6 animals investigated

Fig 2 illustrates the findings from one of the experiments Ejt stimulation evoked potentials of maximum amplitude in the Pcd and the coronal loci (Fig 2 A) Between the loci similar potentials but of lower amplitude were recorded Lower amplitude respon es were also found near the lateral end of the cruciate sulcus but the mapping was not carried far enough laterally in this cat to reveal the point of maximal amplitude in the lateral cruciate region A well defined Ejt locus in area 4 is however shown in Fig 3 A Its maximal point was located near the lateral end of the cruciate sulcu Initially positive surface potentials of lower amplitude were evoked between the 4 locus and the Ejt locus near Pcd

The low thre hold joint projections (Fig 2 A and Fig 3 A) may be compared with those of the group I mu cle afferents (Fig 2 B and Fig 3 C) and the low threshold cutaneous afferents from the contralateral forelimb (Fig 2 C) As described by Oscars on and Rosen (1963 1966) the group I muscle afferents project to Pcd The projection field extends rostrolaterally towards the lateral end of the cruciate sul

Kjt				
Comp I		Comp II		Comp III
Threshold	Ampl max	Threshold	Ampl max	Threshold
16	12	16	13	6
1 00	1 67	1 63	2 78	2 18
	0 33	0 32	0 80	0 40
	1 2–2 5	1 1–2 4	1 7–4 0	1 7–2 8

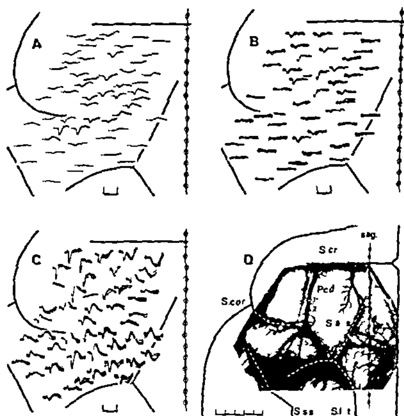


Fig 2 Distribution of cortical surface potentials evoked in the sigmoid and coronal gyri by contralateral low threshold Ejt (A) DR (B) and SR (C) afferents All maps were obtained from the same animal The pattern of the sulci shown on the photograph of the animals brain (D) was transferred to the enlarged diagrams A—C The medial border of these diagrams is related to D by the circle marked saggittal line (Sag) Scales in mm The artefact of each record indicates the recording site Positivity of the evoked potentials is recorded downwards in this and subsequent Figures Cortical points in D 1 the coronal and 2 the Pcd Ejt locus 3 the Pcd DR group I locus 4 the rostal and 5 the caudal SR locus S cru = Sulcus cruciatus S cor = S coronalis S ans = S ansatus S lat = S lateralis S ssa = S suprasylvius anterior Pcd = post cruciate dimple

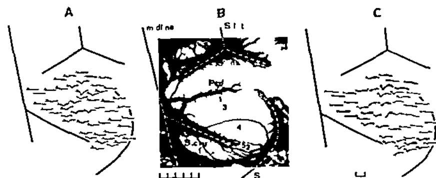


Fig 3 Distribution of cortical surface potentials evoked in the lateral and posterior sigmoid gyrus by contralateral low threshold Ejt (A) and DR (C) afferents The pattern of the sulci of diagrams A and C were transferred from the photograph of the animals brain (B) Area 4γ is delimited in B according to Hassler and Muhs Clement (1964) Cortical points 1 Pcd Ejt locus 2 4γ Ejt locus 3 Pcd DR group I locus 5 4γ DR group I locus

into area 4γ (Fig 3 C) In some animals a well marked locus of large amplitude potentials was found there (*cf* Silfvenius 1968 1972) The low threshold Ejt projections overlapped with those of the muscle afferents in the Pcd and the 4γ loci but the location of the points of maximal amplitude were not identical for the two inputs Maximal Ejt potentials were thus observed caudomedial of the maximal DR point of Pcd in some animals and rostral to this point in others

The projections of the low threshold cutaneous afferents from the contralateral SR analysed by Oscarsson and Rosén (1966) are quite apparent in Fig 2 C showing large initially positive surface potentials in the caudal (CSR) and rostral (RSR) projection areas Although the CSR overlaps the caudo medial part of the coronal Ejt locus the distribution of the projections is obviously not the same because the SR evoked an initially negative surface potential in some of the coronal points where the Ejt response was initially positive

The group I muscle afferents did not evoke responses in the coronal Ejt locus but a small response was occasionally seen in CSR as shown in Fig 2 B

The potentials evoked by SR in area 4γ (RSR) were much larger in amplitude and of shorter latency than those evoked in the Pcd (*cf* Oscarsson and Rosen 1966) There was an overlap between the SR and the Ejt projections both in Pcd and 4γ Rostral to Pcd the low threshold cutaneous afferents evoked a double wave showing early and late positive components Initially negative surface potentials were evoked by SR in the hind limb projection fields of the posterior sigmoid gyrus as well as in the anterior suprasylvian gyrus lateral to CSR

Contributions from the different components of the afferent volley to the potentials in the three cortical Ejt loci

The component I volley due to activation of the lowest threshold Ejt afferents evoked responses in all three of the cortical Ejt loci The amplitudes of the initi

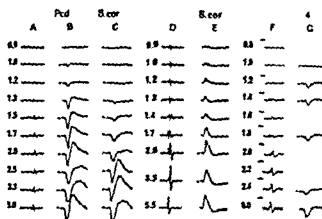


Fig. 4. Peripheral nerve (A, D, F) and cortical (B, C, E, G) potentials evoked by graded electrical stimulation of Fjt. B, cortical potentials evoked in the Pcd and C in the coronal Fjt locus of the same animal. E, in the coronal locus of another animal. G, in area 4γ. B, C and G, cortical surface records. E, microelectrode records 1.3 mm below the surface in the caudal bank of the coronal sulcus. The figures give stimulus strengths in T. Calibration pulse of averaged records (n = 40): 3 ms/50 μV in B, C, G; 5 ms/100 μV in E and 0.3 ms/50 μV in F.

wave of the evoked potentials often reached 25 to 50 per cent of their maxima even before the stimulus strength reached threshold for the component II Fjt volley. The evoked potential then grew with increasing stimulus strength above the maximum of the component I volley. The contribution of component II was observed in all of the three cortical Fjt loci (cf. Fig. 4 and 6 A). A maximal amplitude of the cortical response was sometimes reached with a maximal component II volley, but generally the amplitude grew with increasing stimulus strength due to stimulation of component III afferents.

A component I response was not always evoked in all of the Fjt loci. In the experiment of Fig. 4 A–C such a response was seen in Pcd, whereas the coronal locus only showed component II responses. The depths of the coronal sulcus were not investigated in this animal, but when this was done a component I response was always observed (cf. Fig. 4 D, E).

Time course and latency of the potentials evoked in the cortical Fjt loci

The evoked potentials studied were recorded either from the cortical surface or from deeper layers using microelectrodes. They displayed the usual time course with the

TABLE II. The shortest observed latencies (ms) of the initial component of the cortical Fjt potentials evoked in the Fjt loci at maximal component II strength

	Pcd	Scor	4γ
N	8	5	5
Mean	8.25	6.40	9.80
S.D.	1.58	1.52	2.78
Range	6–10	5–8	7–12

N = number of investigated animals.

Mean, S.D. and range refer to variation between the animals, each of which was represented by the shortest observed latency of each Fjt locus.

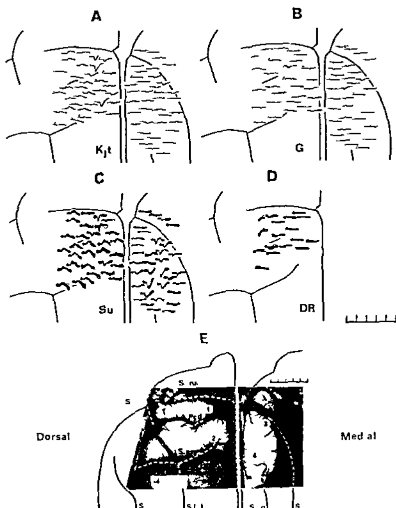


Fig 5 Distribution of cortical surface potentials evoked in the dorsal and medial posterior sigmoid gyrus by the Kjt (A) G (B) Su (C) and DR (D) nerves All maps were obtained from the same animal The pattern of the sulci shown on photograph of the animal brain (E) was transferred to the enlarged diagrams A—D Cortical points in E 1 the dorsal cruciate Kjt locus 2 the ansate Kjt locus 3 the medial cruciate Kjt locus 4 the splenial Kjt locus S p = Sulcus splenialis accessorius other abbreviations as in Fig 1 Scales in mm

initially positive wave at the surface changing to a negative focal potential at 0 2—0 4 mm below the surface The negative potential was maximal at a depth of 1 0 to 1 5 mm This reversal of polarity typical of the evoked response deep in the cortex was observed in all three Ejt loci A distinct thalamocortical volley was rarely observed probably because of the rather small number of fibres involved in the Ejt projections

The latencies of the low threshold Ejt responses in the three Ejt loci are given in Table II and indicate the onsets of the initial deflections in the cortical record

shortest Kjt latencies (5 ms) which were found in the coronal locus are comparable to the shortest latencies of the cortical SR (4.5 ms) and group I DR (5.0 ms) responses obtained in the same experiments. The latencies of the Kjt potentials evoked in the Pcd and area 4γ were 1—2 ms longer than those in the coronal sulcus when compared in the same experiment. The difference between the sample of measurements from the coronal locus on the one hand and those from the Pcd and 4γ on the other was statistically significant at the 5 per cent level.

The cortical topography of the low threshold Kjt projections

Electrical stimulation of the contralateral low threshold Kjt afferents evokes initially positive surface potentials in the posterior sigmoid gyrus medial to the forelimb projections. The Kjt projections showed three or sometimes four loci, two with maximal amplitude on the dorsal aspect and the other one or two on the medial aspect of the hemisphere. The locations of the points of maximal amplitude are indicated by the numbers 1—4 in Fig. 5 E. Cortical point 1 was located in the dorsal projection area of the Group I muscle afferents from the contralateral hind limb. This locus will be referred to as the dorsal cruciate Kjt locus. Point 2 was found within the projection field of the low threshold cutaneous afferents (Su) near the medial end of the medial ansate sulcus. It will be referred to as the ansate Kjt locus. In cases where two separate Kjt loci were observed on the medial aspect of the hemisphere one was located rostrally near the medial projection area of the Group I muscle afferents from the contralateral hind limb (cf. Landgren and Silfvenius 1969). This is called the medial cruciate Kjt locus (point 3 in Fig. 5 E). The other was located more caudally with a point of maximal amplitude near the accessory splenial sulcus. This locus is referred to as the splenial Kjt locus (point 4 in Fig. 5 E).

A response to low threshold Kjt afferents was observed in the dorsal cruciate Kjt locus in 6 of 8 animals and similar responses were found in the ansate Kjt locus in all of 9 animals tested. A distinct division of the Kjt projections to the medial aspect of the hemisphere into a medial cruciate and a splenial locus was seen only in one of 9 animals. The others showed a continuous field that overlapped with the projections from the hind limb group I muscle afferents near the cruciate sulcus. In those cases the point of maximal amplitude in the Kjt field was located caudal to the maximum point of the muscle afferents (thus about halfway between figures 3 and 4 in Fig. 5 E). Because of the overlap with the projections of the group I muscle afferents these Kjt loci were classified as medial cruciate and not as splenial Kjt loci in Table III.

The cortical Kjt loci were related to the projection fields and points of maximal amplitude of the low threshold muscle and cutaneous afferents from the contralateral hind and forelimbs. These relations are illustrated in Fig. 5 A—D. The maps were obtained from the brain photographed in Fig. 5 E. The four Kjt loci were well separated in this animal. A band of initially negative surface potentials thus separated the two Kjt loci on the dorsal surface of the posterior sigmoid gyrus. In most brains

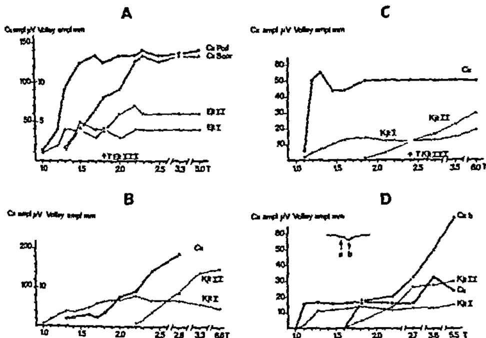


Fig 6 Correlation between stimulus strengths amplitudes of the components of the afferent volley in the peripheral nerve and amplitudes of the cortical evoked potentials I II and III refer to the components of the Ejt and Kjt volleys A cortical evoked potentials (Cx) recorded from the Pcd Ejt locus (Cx Pcd) and the coronal Ejt locus (Cx S cor) B Cx from the dorsal cruciate Kjt locus C Cx from the splenial and D from the medial cruciate Kjt loci The inset in D shows an original record of the cortical potential with two components (a and b)

however a separation with negative surface potentials was found only near the mid line and the potentails more laterally were initially positive

A comparison of the maps A and B of Fig 5 shows that the dorsal and medial cruciate Kjt loci were situated within the projection fields of the group I gastrocnemius afferents The points of maximal amplitude were however not identical The dorsal cruciate Kjt locus and the dorsal group I hind limb locus of G were found at the medial border of the group I forelimb area near the Pcd The overlap between fore and hind limb projections was limited to a narrow zone (Fig 5 A B and D) The ansate and the splenial Kjt loci both coincide with the maximal points of the low threshold Su projections (Fig 5 A and C) Only insignificant responses were evoked by group I muscle afferents in these two loci

Contributions from the different components of the afferent volley to the potentials evoked in the cortical Kjt loci

The lowest threshold fibres in the Kjt those producing the component I of the afferent volley projected to all of the Kjt loci described above Generally an increase in amplitude was added to the evoked potential when the afferents of component II

TABLE III. The shortest observed latencies (ms) of the initial component of the cortical Ejt potential evoked in the Ejt loci at maximal component II strength

	Dors cruciate Ejt	Med cruciate Ejt	Ansate Ejt	Splenial Ejt
N	7	9	10	1
Mean	10.57	13.11	11.65	13
S.D.	1.81	2.20	2.24	
Range	8–13	10–16	8–15	

Abbreviations as in Table II

were also discharged (cf. Fig. 6 B). Some cortical points were, however, observed where the evoked response did not increase in amplitude when the stimulus strength was raised above the maximum of component I. Such a case is illustrated in diagram C of Fig. 6. The records were obtained from the splenial Ejt locus. Under the existing experimental conditions that particular cortical locus thus seemed to receive only component I projections.

Time course and latency of the potentials evoked in the cortical Ejt loci

The cortical potentials evoked by the low threshold Ejt afferents showed the well known time course with an initially positive deflection recorded at the cortical surface that changed to initially negative potential in the deeper cortical layers. This pattern was confirmed in all the described cortical Ejt loci. Initially positive surface potentials rising in two steps were sometimes observed (cf. inset of Fig. 6 D) but this phenomenon could not be ascribed to any particular locus. A thalamo-cortical Ejt volley was seen as a small initial deflection in just a few of the evoked potentials.

The latencies of the Ejt responses in the different cortical loci are given in Table III. Latencies as short as 8 ms were found in the dorsal cruciate and ansate Ejt loci and such values were comparable with the shortest latencies produced there by the low threshold cutaneous afferents. In contrast, the Ejt latencies on the medial surface of the hemisphere were slightly longer, the shortest being 10 ms. The difference between the mean latencies of the samples obtained from the dorsal and the medial cruciate Ejt loci was statistically significant at the 5 per cent level.

The spinal course of the projections from the low threshold joint afferents

The Ejt projections. The spinal course of the projections from the low threshold Ejt afferents was studied in 6 animals. It was found in 3 cases that all potentials evoked by both components I and II of the Ejt volley disappeared after transsection of the dorsal columns at cervical levels C_1 to C_4 (Fig. 7 B). In 3 other animals some response remained indicating that at least a portion of the ascending Ejt path must lie outside the dorsal columns. One of these experiments is illustrated in Fig. 7 A and C, which shows that although the cortical response in the Pcd and the coronal loci were normally abolished by dorsal column transection when tested with a single Ejt

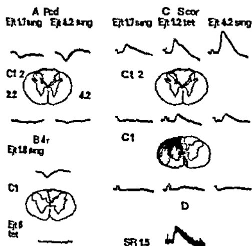


Fig 7 The effect of spinal cord lesions on the cortical potentials evoked by low threshold Ejt afferents in Pcd (A) 4γ (B) and coronal (C) Ejt loci A and B surface potentials C microelectrode records at 1 0 mm below the surface Control records above and records obtained after the lesions below the diagrams of the cervical cord The extent of the lesion is indicated by the hatched area Cervical level is given to the left of the diagram D Cortical response evoked by SR in the coronal locus after all lesions Stimulus strength in T used to evoke the response is shown by the figures near the records Sing single shock tet train of 3 shocks at 600 Hz Calibration pulse 3 ms/50 μV

volley, a short train of even smaller volleys was still able to produce a response in the coronal locus The remaining response disappeared after a spinal hemisection at C_1 contralateral to the stimulated nerve

In another experiment the potential evoked in the coronal locus by an Ejt component I volley was abolished after cervical dorsal column transsection but a response was still obtained with the component II volley The third animal showed an Ejt component II response in the Pcd 4γ and coronal loci The Pcd and 4γ potentials were reduced in amplitude but did not disappear after a dorsal column transsection between C_3 and C_4 In the coronal Ejt locus however the initially positive surface potential changed to an initially negative potential with a latency 4 ms longer

The main conclusion to be drawn from these experiments is that the major portion of the low threshold Ejt path lies in the dorsal columns although additional paths outside the dorsal columns apparently do exist and they cross the midline below C_1

The Kjt projections The spinal course of the ascending projections from Kjt was investigated in 8 cats The main findings suggest there are two ascending paths for the low threshold Kjt components one located in the dorsal column (*DC*) and the other in the dorsolateral fascicle (DLF) Fig 8 A—B D—E and H—I shows that the potentials evoked in the dorsal and medial cruciate Kjt loci and in the ansate Kjt locus were reduced in amplitude by a DC transsection A subsequent cut in the DLF made superficially at C_1 on the side of the stimulated nerve abolished the remaining Kjt response (Fig 8 B—C and E—F Similar results were obtained when the transsection of the DLF preceded that of the DC (Fig 8 G—L) Only high threshold Kjt potentials remained after the two transsections (Fig 8 I) The potentials evoked by BSt in Fig 8 E by SR in F and DR in L serve as controls indicating that the cerebral cortex was not depressed by the lesions

Further analysis of the eight experiments showed that in four of them the cortical

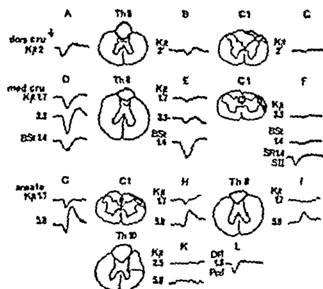


Fig 8 The effect of spinal cord lesions on the cortical surface potentials evoked by low threshold Kjt afferents in the dorsal cruciate (A—C) the medial cruciate (D—F) and the ansate (G—K) Kjt loci Control records to the left and records obtained after the lesions (hatched) to the right of the spinal cord diagrams Stimulus strength used is given in T for each record Potentials evoked by SR in S II (F) and by DR in Icd (L) were recorded after all lesions The first shock artefact coincides with the end of the calibration pulse in all records except A where it is indicated by an arrow Kjt train of 3 shocks 600 Hz BSt DR SR single shocks Calibration pulse 3 ms/50 μV

response evoked by the component I of the Kjt was abolished by a transsection of the DC alone However the component II still produced a response though with a latency which was 2—3 ms longer than that of the control This component II response was then abolished by transsecting the DLF at C_1 on the side of the stimulated afferents These observations were made in the dorsal and medial cruciate and the ansate Kjt loci

In a fifth experiment only a component I response was evoked in the splenial Kjt locus (cf Fig 6 C) This response was not influenced by a transsection of the DLF at C_1 on the side of the stimulated afferents It was however abolished by a subsequent transsection of the DC at Th 10

The results of these five experiments lead to the conclusion that the projections from the component I of Kjt travel in the DC and those from component II in the DLF The transsection of the DLF was made at the level of C_1 thus above the lateral cervical nucleus This lesion always abolished the cortical response evoked by the group I muscle afferents from the hind limb (cf Fig 8 E and F) The component II projections of Kjt therefore accompanies the low threshold muscle afferent at least up to the level of C_1 and this hind limb joint path can accordingly not be identical with the spino cervico thalamic tract

Although the above conclusions are justified for five of our eight experiments they can not be generalized because a more complex pattern emerged from the remaining three In one of these a component I response evoked in the medial cruciate Kjt locus was reduced to 20 per cent by the transsection of the DC A subsequent cut in the DLF at C_1 abolished the entire response to components I and II This observation thus indicates that some of the projections from Kjt component I may

travel in the DLF The same conclusion was drawn from another experiment in which a cortical component I potential was found to disappear after a transection of the DLF at C_1 In the third experiment it was found that the cortical response evoked in the ansate Kjt locus by Kjt component II was reduced though not abolished by the transection of the DLF at C_1 The remaining component II potential was then abolished by a transection of the DC at Th 8 According to this experiment some of the component II projections did ascend in the DC Considering all our evidence it is therefore concluded that the Kjt component I projections travel mainly in DC and the component II mainly in DLF but some contribution from component II may be found in DC and some component I in DLF

Discussion

In this study the projections of the joint afferents to the cerebral cortex were described in terms of specific loci *i e* circumscribed cortical areas within which evoked potentials were recorded The amplitude of the potentials were maximal in the center of the area and declined gradually towards its borders This pattern presumably implies that the projections discharge a dense population of the cortical neurones near the center of the locus whereas the density of the connections decreases towards the periphery The term locus is therefore roughly equivalent to the *discharge zone* as defined by Mountcastle (1957)

The loci were found to occupy the same cortical sites in several investigated animals They are therefore assumed to indicate excitatory connections available to the low threshold joint afferents in the chloralose animal

The cortical evoked potentials generally increased in amplitude with increasing stimulus strength throughout the range of components I and II Apparently therefore component I (probably Paciniform or Golgi) as well as component II (predominantly Ruffini) afferents contribute to the projections to all cortical loci Exceptions from this rule were observed however and experiments with natural stimulation of the receptors will be required to settle questions concerning the contributions from specific receptor types to the responses evoked in the different cortical loci

Several investigators have described cortical units responding to joint movements and both slowly and rapidly adapting responses have been seen However when movements of joints are used to evoke the central discharge it is impossible to tell whether the receptors involved are located in muscles tendons joint capsules or ligaments Responses evoked by electrical stimulation of specific joint nerves thus provide additional evidence to complement results obtained using natural stimulation It is of interest therefore to compare the present results with those obtained from experiments using natural stimulation

Mountcastle (1957) described a population of 105 cortical cells activated by movement of the forelimb These cells were located in the posterior sigmoid gyrus of the cat between the Pcd and the junction between the ansate and lateral sulci According to his observation the probability of encountering deep joint units in

crea ed as the penetrations approached the Pcd This agrees with our present findings of an Ejt locus near Pcd There is also a general agreement between the feline postcruciate joint projections and the corresponding postcentral joint projections in the monkey Powell and Mountcastle (1959) thus described a heavy projection from deep tissues to area 3 a in the depth of the central sulcus of the monkey Further posterior area 3 was dominated by cells activated by cutaneous stimuli As the recording site was moved away from the central sulcus the number of response to joint movements increased again particularly in the area 2 A similar organization has now been observed in the cat where cortical potentials evoked by low threshold muscle and joint afferents near Pcd (corresponding to area 3 a) decrease in amplitude and finally disappear as the recording site is moved further caudally between the Pcd and the an ate sulcus (area 3 b and 1) The projections in the monkey from deep tissues to the area 2 in fact correspond to a projection in the cat from group I muscle afferents to the rostral bank of the lateral ansate sulcus as described by Silfvenius (1968) However this area was not tested in the present investigation and it is therefore not known whether joint afferents do indeed project to the ansate sulcus

In the cat cortical cells discharged by joint movements were found in the posterior sigmoid gyrus between the Pcd and the cruciate sulcus by Brooks Rudomin and Slayman (1961) Similar cells near the lateral end of the cruciate sulcus were also described by Baker Tyner and Towe (1971) Our finding of an Ejt locus in area 4 γ near the lateral end of the cruciate sulcus confirms their observations The precoronal recording site described by Baker Tyner and Towe (1971) is obviously not identical with our coronal Ejt locus in the posterior bank of the coronal sulcus Accordingly nly 3 per cent of their precoronal units responded to joint movements

In the present investigation the localization of th low threshold joint projections was referred to that of the low threshold muscle and cutaneous afferents The locations were also compared to the cytoarchitectonic areas described by Hassler and Muhs Clement (1964 on the basis of the patterns formed by the sulci This method does not allow for individual variations of the extent of the cytoarchitectonic areas However the localization near Pcd of the projections from the low threshold afferents has been confirmed in a large number of cats and the cytoarchitectonical details of the cortex near a microelectrode track left in the maximum point of DR was investigated in some of them (Landgren and Silfvenius unpublished) The cytoarchitecture fitted the criteria given for the area 3 a by Hassler and Muhs-Clement (1964) The cortex surrounding a microelectrode track placed in the maximum point of Su near the medial end of the an ate ulcus showed the features of the area 1 As mentioned above the ansate Kjt locus overlaps with the Su projections in this region In the monkey the cytoarchitecture of the area 3 a was found in the cortical projection field of the large muscle spindle afferents by Phillips Powell and Wiesendanger (1971) The reference of the electrophysiological observations to particular cytoarchitectonic areas therefore eems justified

A zonal convergence of projections from low threshold joint muscle and skin

afferents was observed in area 3 (the Pcd locus of Ejt the dorsal and the medial cruciate Kjt loci) and in area 4 γ near the lateral end of the cruciate sulcus The motor cortex proper as well as its border zone thus receive short latency information from three different sets of receptors discharged by movements of the limbs

The joint loci of the areas 1 and 2 (the coronal Ejt locus and the ansate Kjt locus) overlapped with the cutaneous projections but showed no responses to activation of group I muscle afferents This difference in afferent input may indicate a functional difference between the joint projections to area 3 on the one hand and those to area 1 and 2 on the other hand The observed differences in the latency of the responses evoked in the various loci also suggest a functional differentiation The nature of the function is at present open to speculations

The distinction between the joint loci of area 3 and those of area 1 and 2 is at variance with the somatotopic maps of the cat's cerebral cortex developed by Woolsey (1958)

The cytoarchitectonic organization of the cat's posterior sigmoid gyrus was not known at that time and the significance of Pcd as analogous to the depth of the central sulcus in the primates was not appreciated The digits of the sensory and motor figurines on the maps met at the cruciate sulcus and a single sensory map covered all of the posterior sigmoid gyrus The present results provide evidence of a more detailed organization with recognition of a separate sensory input to area 3 The somatotopic organization within areas 1 and 2 was not investigated in detail in the present series of experiments but our findings are in agreement with this established concept

The findings of Kjt loci on the dorsal as well as on the medial aspect of the posterior sigmoid gyrus confirm the previous results of Woolsey (1947) His idea of a preaxial representation on the dorsal aspect of the hemisphere and a postaxial one on the medial aspect can however not be supported because afferents from the posterior part of the joint capsule (*cf* Freeman and Wyke 1967) were found to project to the dorsal as well as to the medial aspect Low threshold afferents from preaxial and postaxial muscles also project both to the dorsal and to the medial aspect of the hemisphere (*cf* Landgren and Silfvenius 1969)

Burgess and Clark (1969 a) and Clark (1972) have studied the spinal projections of the primary afferent fibres from the Kjt They found that low threshold component I fibres innervate both rapidly and slowly adapting receptors Their studies of the conduction velocities of the primary afferents also showed some overlap between rapidly and slowly adapting fibres in the component II range Only the rapidly adapting receptors projected to cervical levels via DC

Our results agree with these observation The cortical potentials evoked through the DC appeared most often with component I of the Kjt but a contribution from component II was observed in one case of eight These cortical responses ought to be due to impulses from rapidly adapting joint receptors mainly with large axons On the other hand the cortical responses to component II were evoked via DLF in all the animals tested Two of these showed in addition evidence of a component

I response that depended upon the DLF These findings indicate that the DLF carries impulses to the cortex mainly from the slowly adapting Ruffini endings which are included in the component II but also from component I afferents presumably originating in slowly adapting Golgi receptors

The low threshold joint projections in the DLF ascend uncrossed to the level of C_1 Transecting the DLF at this level abolishes the cortical potentials evoked by both the hind limb group I muscle afferents and the low threshold joint contribution that remains after a preceding DC transection The paths from the muscles and joints may therefore have similar courses in the spinal cord Lindström and Takata (1972) have described excitation of dorsal spinocerebellar tract neurones by Kjt component I and II volleys It is possible that the joint path in the dorsolateral fascicle could be subserved by collaterals of the dorsal spinocerebellar tract but further experiments are required to establish this point

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References

ANDERSEN H T L KORNER S LANDGREN and H SILFVENIUS Fibre components and cortical projections of the elbow joint nerve in the cat Acta physiol scand 1967 69 373-382
ANDREW B L The sensory innervation of the medial ligament of the knee joint J Physiol Lond 1954 123 241—250
BAKER M A C F TYNER and A L TOWE Observations on single neurones recorded in the sigmoid gyri of awake nonparalyzed cats Exp Neurol 1971 32 3 8—403
BOYD I A The histological structure of the receptors in the knee joint of the cat correlated with their physiological response J Physiol (Lond) 1954 124 476—488
BROOKS V B P RUDOMIN and C L SLAYMAN Sensory activation of neurone in the cats cerebral cortex J Neurophysiol 1961 24 78 301
BURGESS P R and F J CLARK Dorsal column projection of fibres from the cat knee joint J Physiol Lond 1969a 203 301—315
BURGESS P R and F J CLARK Characteristics of knee joint receptors in the cat J Physiol Lond 1969b 203 31 —335
CLARK F J Central projections of sensory fibres from the cat knee joint J Neurobiol 19 2 3 101—110
FREEMAN M A R and B WYKE The innervation of the knee joint An anatomical and histological study in the cat J Anat 196 101 505—532
GARDNER E The distribution and termination of nerves in the knee joint of the cat J comp Neurol 1944 80 11—32
HASSLER R and K MUHS-CLEMENT Architektonischer Aufbau des sensomotorischen und parietalen Cortex der Katze J Hirnforsch 1964 6 3 7—420
KLUVER H and E BARRERA A method for the combined staining of cells and fibres in the nervous system J Neuropath exp Neurol 1953 12 400—403
KORNER L and S LANDGREN Projections of low threshold joint afferents to the cerebral cortex of the cat Acta physiol scand 1969 76 5A—A
LANDGREN S and H SILFVENIUS Projection to cerebral cortex of Group I muscle afferents from the cats hind limb J Physiol Lond 1969 200 353—37
LINDSTROM S and M TAKATA Monosynaptic excitation of dorsal spinocerebellar tract neurones from low threshold joint afferents Acta physiol scand 1972 84 430—432
MOUNTCASTLE V B Modality and topographic properties of single neurones of cats somatic sensory cortex J Neurophysiol 1957 20 408-434
OSCARSSON O Three ascending tracts activated from Group I afferents in forelimb nerves of the cat Prog Brain Res 1964 12 179—196
OSCARSSON O and I ROSEN Projection to cerebral cortex of large muscle spindle afferents in forelimb nerves of the cat J Physiol Lond 1963 169 924—945
OSCARSSON O and I ROSEN Short latency projections to the cats cerebral cortex from skin and muscle afferents in the contralateral forelimb J Physiol (Lond) 1966 182 164—184

PHILLIPS C G T P S POWELL and WIESENDANGER, Projection from lo threshold muscle afferents of hand and forearm to area 3a of baboon s cortex *J Physiol* (Lond) 1971 *217* 419—446

POWELL T P S and V B MOUNTCASTLE Some aspects of the functional organization of the cortex of the postcentral gyrus of the monkey A correlation of findings in a single unit analysis with cytoarchitecture *Bull Johns Hopkins Hosp* 1959 *105* 133—162

SILFVENIUS H Cortical projections of large muscle afferents from the cat s forelimb *Acta physiol scand* 1968 *74* 25A—26A

SILFVENIUS H Projections to the cat cerebral cortex from fore and hind limb Group I muscle afferents *Umed Univ Med Diss* 1972 No 4

SKOGLUND S Anatomical and physiological studies of knee joint innervation of the cat *Acta physiol scand* 1956 *36* Suppl 124

WOOLSEY C N Patterns of sensory representation in the cerebral cortex *Fed Proc* 1947 *6* 437—441

WOOLSEY C N Organization of somatic sensory and motor areas of the cerebral cortex *Biological and biochemical basis of behaviour* Ed Harlow H F and C N Woolsey Univ Wisconsin Press Madison 1958

Acta physiol scand 1973 89 522—530

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A Study on the Action of Noradrenaline on Ionic Content and Sodium, Potassium and Chloride Effluxes in the Rat Portal Vein

By A. Wahlström*

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Abstract

Wahlström B A. *A study on the action of noradrenaline on ionic content and sodium potassium and chloride effluxes in the rat portal vein.* Acta physiol scand 1973 89 522—530

The effects of noradrenaline on the sodium, potassium, chloride and calcium [illegible]ntent were studied. It was found that incubation in noradrenaline solution [illegible] 6 [illegible] M) for 30 min caused a decrease in Cl content from 86 mmol/kg wetweight to 80 mmol/kg wetweight while the Ca content increased from 4 1 to [illegible] mmol kg wet[illegible]eight. There were no changes in [illegible] or K content.

When noradrenaline [illegible] 6 [illegible] M was added during the washout of [illegible]Cl the rate of flux of Cl increased by 8[illegible]. This effect was dose-dependent in the range 0 06 to 6 [illegible] M while a higher concentration had a le[illegible] than maximal effect on the rate of flux. Noradrenaline increased the rate of flux of [illegible]K as well but this effect was small 15—20 [illegible] and independent of dose. There [illegible] no effect of the drug on [illegible]Na efflux. Flux data were used to c[illegible]lation permeabilities and membrane potential in the presence of noradrenaline taking into account the depolarization caused by the drug. The ratio P_K [illegible] was 1 0 063 3[illegible] and the predicted membrane potential [illegible] mV. The membrane potential recorded by [illegible]cellular microelectrodes was [illegible] mV during noradrenaline stimulation. The shown effects of noradrenaline were blocked by phenoxybenzamine while propranolol had no effect. It [illegible] concluded that the change in chloride permeability can satisfactorily explain the depolarization action caused by noradrenaline.

Noradrenaline contracts rat portal vein through depolarization and increased frequency of electrical discharge (Axelsson *et al* 1966). This effect is markedly reduced in Ca free or Ca low solutions. Evidence for Ca being involved in catecholamine action in intestinal smooth muscle was given by Bulbring and Tomita (1969 b). In the taenia coli of the guinea pig the action of noradrenaline and adrenaline is mediated via a specific increase in potassium permeability (Huter Bauer and Goodford 1963, Bulbring Goodford and Setekleiv 1966, Bulbring and Tomita 1969 a). Wahlström (1971) found no change in potassium efflux when the rat portal vein was stimulated by noradrenaline. On the other hand in rabbit carotid artery it has been

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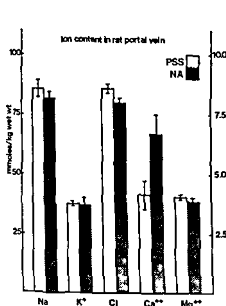


Fig 1

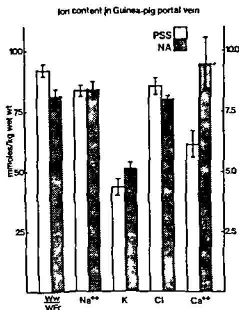


Fig 2

Fig 1 The ionic content and W_w/W_f ratio of rat portal vein in normal PSS (empty columns) and aft r 30 min in normal PSS + 6 μM noradrenaline (filled columns) Vert cal bars indicate $\pm$S E (n = 6) An asterisk indicates that the d fference between means is significant $p < 0.05$ Left vertical axis mmol/kg W_w for Na and K and for W_w/W_f rat o Right vertical axis mmol/kg W_w for Ca^{++} and Mg

Fig 2 The ionic content and W /W_f ratio of guinea pig portal ein in normal PSS (empty columns) and after 30 min in normal PSS + 6 M noradrenal ne (filled columns) Vertical bars ind cate $\pm$S E (n = 8) An asterisk i dicates a significant diffe ence between means $p < 0.05$ Left and right vertical axes as in Fig 1

shown that adrenaline increases Cl conductance (Mekata and Niu 1971) Since the K and Cl distribution and permeability are equally important in determining the resting potential and membrane conductance in the rat portal vein (Wahlstrom 1973) this study was undertaken to investigate the possibility that noradrenaline stimulates the portal vein through specific ion permeability changes Some preliminary results have been presented to the Physiological Societ Meeting in Cambridge July 1972 (Wahlstrom 1972)

Methods

The general procedu e i d scribed in a accomp nying paper (Wahlst om 1 73) and only differences from those methods w ll be gi n here

Ion content and ECS expe ments Muscles were d ded into two group one wh ch was incubated in normal PSS for a certa n per od of time and another wh h was ncubated f r the same length of time in normal PSS followed by ncubat on in a olut on ontain ng noradrenal ne For determ nat ons of e tracellular p ce (ECS) both g o ps w incubated in solutions contain ng [60] CoEDTA for 10 m n at the nd of the i ubation period

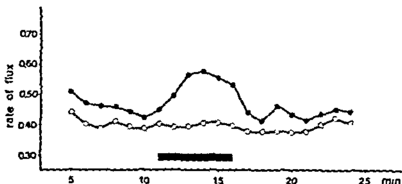


Fig. 5. The effect of noradrenaline on the rate of flux of ^{42}K in normal PSS (filled circles) and in normal PSS + 6 μM phenoxybenzamine (open circles). Noradrenaline 6 μM was applied for 6 min as shown by the black bar. Abscissa: Time in min. Ordinate: Rate of flux of ^{42}K defined as in Fig. 3 in arbitrary units.

The results from 24 muscles tested at 4 different concentrations of noradrenaline are summarized in Fig. 4. The effect was maximal at 6 μM being 185.3 ± 20.0 % of normal efflux. A 10 times lower concentration had a very slight effect, i.e. 111.0 ± 1.4 at 0.6 μM. A higher concentration, in this case 60 μM, had a less than maximal effect: 148.5 ± 8.4 %.

It could not be excluded that the increase in the rate of chloride efflux in the presence of noradrenaline was secondary to the excitation caused by the drug and not due to the drug primarily changing chloride permeability. To test this possibility the excitatory effect of noradrenaline was mimicked by increasing the external potassium concentration to 24 mM for 5 min during the washout of ^{36}Cl. This treatment had no effect on the rate of efflux of ^{36}Cl although it causes a depolarization of several millivolts and greatly increases the frequency and magnitude of the spontaneous contractions.

Ca and the effect of noradrenaline on ^{36}Cl efflux. Exposure to noradrenaline was found greatly to increase the calcium content of the portal vein. Therefore experiments were performed in which the [Ca^{++}] was varied from 0.1 to 10 mM and the effect of noradrenaline tested in these conditions. The changed Ca concentration had no effect in itself on the ^{36}Cl efflux nor on the total chloride content. The rate constant for the slow component was the same for both 0.1 and 10 mM Ca. On the other hand, when noradrenaline 10 μM was added during the efflux there was no change in the rate of flux in the Ca low solution while the drug increased the rate of flux in the Ca high solution by 57 ± 7 % (n = 6).

The effects of noradrenaline on ^{42}K efflux. Muscles loaded for three h in ^{42}K solution were mounted in the perfusion chamber, the washout started and noradrenaline added for 5 min during the slow phase of the washout. Fig. 5 (filled circles) shows the rate of flux of ^{42}K in an individual muscle during such an experiment. When noradrenaline 6 μM was added the rate of flux increased by roughly

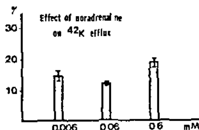


Fig 6 The effect of different concentrations of noradrenaline on the rate of ^{42}K efflux Abscissa Concentration of noradrenaline in mM Ordinate Percentage increase in the rate of ^{42}K efflux Vertical bars indicate ±S E (n = 6)

15% (mean increase over the total exposure period) The same muscle was reloaded and the experiment repeated in the presence of 6 μM phenoxybenzamine (open circles in Fig 5) In these conditions noradrenaline had no effect on the rate of flux Fig 6 summarizes the result for 18 muscles at three different concentrations The effect of noradrenaline was not dose dependent in the range 6 μM to 0 6 mM Lower concentrations were not tried for the reason that changes in the rate of flux would be small and difficult to detect

It seemed highly probable that the effect of noradrenaline on ^{42}K efflux was caused by the excitation itself since it was not dose dependent and small even at very large concentrations of the drug Experiments were therefore performed in K high and Ca low solution In the K high solution noradrenaline affects only the tension while in Ca low solution both the electrical and mechanical response is diminished or completely abolished In both these types of solutions noradrenaline 10 μM had no effect on the efflux of ^{42}K Furthermore increasing the [K]$_o$ only moderately to 24 mM (Wahlstrom 1973) had a much larger effect on the ^{42}K efflux than had a dose of noradrenaline which gave a similar electrical and mechanical response

The effect of noradrenaline on ^{24}Na efflux Noradrenaline 6 μM when added during the washout of ^{24}Na had no effect on the shape of the efflux curve nor did it change the rate of flux This is not surprising since the passive Na movements are directed inwards and the outward movements are the result of the activity of the Na K pump which does not seem to be influenced by noradrenaline



Fig 7 The effect of noradrenaline 6 μM on membrane potential and electrical activity recorded intracellularly by microelectrodes Noradrenaline was added to the perfusion solution at the left arrow and removed at the right arrow Note complete cessation of electrical discharge during maximum depolarization The uppermost straight line is the zero potential Time and voltage scales are given by the horizontal and vertical bars

TABLE 3

	Na	K	Cl
Extracellular ion concentration (mM)	137.5	6.0	134.1
Intracellular ion concentration (mM)	13.0	197.7	64.0
Permeability (cm/sec × 10⁻)	0.175	2.79	9.67
Calculated membrane potential (mV)		−27.55 (n = 8)	

Membrane permeabilities and membrane potential in the presence of noradrenaline The equations used to calculate permeabilities and membrane potential are given in an earlier paper (Wahlstrom 1973) When noradrenaline 6 μM was added to the perfusion solution the membrane was depolarized by 20—25 mV during 1—2 minutes as shown in Fig 7 The lowest membrane potential during the action of the drug was -25 ± 3 mV ($n = 4$) The permeabilities for sodium potassium and chloride during maximum noradrenaline effect were then calculated taking into account the depolarization caused by the drug and the change in the rate of flux It was also assumed that during these short exposure times there were no net movements of the respective ions Theoretically the Na and K effluxes ought to increase as a result of the depolarization while for Cl a decrease in efflux was expected Since the Cl efflux actually increased when the tissue was exposed to noradrenaline the estimated change in Cl permeability becomes very large when the depolarization is taken into account The calculated permeabilities of sodium potassium and chloride and the calculated membrane potential in the presence of noradrenaline are given in Table 3 The permeability ratio P_K P_{Na} P_{Cl} was 1 0.034 0.816 in normal solution (Wahlstrom 1973) at a membrane potential of −45 mV while in the presence of noradrenaline it was 1 0.063 3.85 The calculated membrane potential of −27.55 mV comes very close to the measured value of −25 mV The measured changes in [36]Cl efflux in response to noradrenaline thus satisfactorily explain the change in membrane potential

Discussion

In intestinal smooth muscle there is evidence that conductance changes towards Na K and even Ca are involved in the α action of catecholamines (Bulbring and Kuriyama 1963 Bülbring Goodford and Setekleiv 1966 Jenkinson and Morton 1967) The only indication of chloride being involved was put forward by Bulbring and Tomita (1969) and Ohashi (1971) The former authors stressed that the change in K conductance is more important than that in Cl conductance in determining the response of the tissue to noradrenaline Ohashi calculated the relative contribution of Cl to the increase in membrane conductance caused by adrenaline to 36 % These tissues show *inhibitory* responses to catecholamines On the other hand Mekata and

Niu (1971) stated that the excitatory α effect of adrenaline on the rabbit common carotid artery was due to an increase in both Na and Cl conductance Bulbring and Szurszewski (1971) concluded that a large proportion of the excitatory response to catecholamines in guinea pig uterus could be ascribed to an increase in Cl con ductance The present results show that in the rat portal vein noradrenaline causes an increase in the chloride efflux and that this effect is associated with a depolariza tion of the membrane Noradrenaline also causes a decreases in the Cl content during longer exposure times These findings together with the good agreement be tween the predicted and measured membrane potential in the presence of nor adrenaline strongly suggest that the excitatory action of noradrenaline in the rat portal vein is mediated by an increase in P_{Cl} The effects of noradrenaline on ion flux are completely blocked by phenoxybenzamine while propranolol has no effect Thus by convention the shown effects represent the α action of noradrenaline

In an earlier paper (Wahlstrom 1971) we reported that noradrenaline had no effect on the K efflux This proved not to be strictly true However the effect of the drug on K efflux was small and when the depolarization caused by noradrenaline was taken into account there was actually a slight decrease in P_K The small change in efflux may thus be regarded as a result of an increased membrane activity

During exposure to noradrenaline the Ca content of the tissue increased contrary to findings from rabbit aortic smooth muscle where Van Breemen *et al* (1972) showed that there was no increase in Ca uptake when noradrenaline was added However Johansson *et al* (1967) have shown that the maximum tension develop-ment of the rat portal vein increases with time during prolonged exposures to nor adrenaline Therefore it eems likely that there exists a relationship between the total Ca content of the tissue and the amount of Ca released intracellularly during a burst and that this is influenced by noradrenaline That noradrenaline might also release intracellular Ca was suggested by Axelsson *et al* (1966) This was confirmed for rabbit aorta by Van Breemen *et al* (1972) Finally it was found that the effects of noradrenaline on the rate of chloride efflux were abolished in Ca deficient solution Experiments to elucidate the different roles of Ca in the response of the rat portal vein to noradrenaline are in progress

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References

AXELSSON J B JOHANSSON O JONSSON and B WAHLSTROM The effects of adrene gic drugs on electri al and mechanical activity of the rat po tal e n *B bl anat* (Basel) 1966 *8* 16—20

BRADING A F Analysis of the effluxes of sodium potassium and chloride ion from smooth muscle in normal and hypertonic solution *J Phys l* (Lond) 1971 *214* 393—416

BULBRING E P J GOODFORD and J SETEKLEIV The action of adrenaline on the ionic content and sodium and potassium movements in the smooth muscle of the guinea pig taenia coli Brit J Pharmacol 1966 28 296—307

BULBRING E and H KURIYAMA Effects of changes in the ionic environment on the action of acetylcholine and adrenaline on the smooth muscle cells of guinea pig taenia coli J Physiol (Lond) 1963 166 59—74

BULBRING E and J SZURSZEWSKI The excitatory action of noradrenaline (α action) on guinea pig myometrium compared with that of acetylcholine J Physiol (Lond) 1971 217 39—41P

BULBRING E and T TOMITA Increase of membrane conductance by adrenaline in the smooth muscle of guinea pig taenia coli Proc roy Soc B 1969a 172 89—102

BULBRING E and T TOMITA Effect of calcium barium and manganese on the action of adrenaline in the smooth muscle of the guinea pig taenia coli Proc roy Soc B 1969b 172 121—136

HUTER J H BAUER and P J GOODFORD Die Wirkung von Adrenalin auf Kalium Austausch und Kalium Konzentration im glatten Muskel (Taenia Coli des Meerschweinchens) Naunyn-Schmiedebergs Arch exp Path Pharmak 1963 246 75—76

JENKINSON D H and I K M MORTON Effects of noradrenaline on the permeability of depolarized intestinal smooth muscle to inorganic ions J Physiol (Lond) 1967 188 373—386

JOHANSSON B O JONSSON J AXELSSON and B WAHLSTROM Electrical and mechanical characteristics of vascular smooth muscle response to norepinephrine and isoproterenol Circulat Res 1967 21 619—633

MEKATA F and H NIU Biophysical effects of adrenaline on the smooth muscle of the rabbit common carotid artery J gen Physiol 1971 59 92—102

OHASHI H The relative contribution of K and Cl to the total increase of membrane conductance produced by adrenaline on the smooth muscle of guinea pig taenia coli J Physiol (Lond) 1971 212 561—575

VAN BREEMEN C B R FARINAS P GERBA and E D MCNAUGHTON Excitation contraction coupling in rabbit aorta studied by the lanthanum method for measuring cellular calcium influx Circulat Res 1972 30 44—54

WAHLSTROM B The effects of changes in the ionic environment on venous smooth muscle distribution of sodium and potassium Acta physiol scand 1971 82 382—392

WAHLSTROM B The effects of noradrenaline on ion content and ^{42}K and ^{36}Cl effluxes in the rat portal vein J Physiol (Lond) 1972 226 63—64P

WAHLSTROM B Ionic fluxes in the rat portal vein and the applicability of the Goldman equation in predicting the membrane potential from flux data Acta physiol scand 1973 89 436—448

Acta physiol scand 1973 89 531—537
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Autoregulation of Blood Flow in Human Adipose Tissue

By

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Abstract

HENRIKSEN O S L NIELSEN and W P PAASKE *Autoregulation of blood flow in human adipose tissue* Acta physiol scand 1973 *89* 531—537

The presence of autoregulation of blood flow was investigated in subcutaneous adipose tissue of the distal part of the forearm by means of the local xenon 133 washout technique 89 studies were performed on 4 healthy subjects Blood pressure changes were obtained by passive displacement of the investigated area to various test levels above and below the reference level the jugular notch Above heart level where venous pressure was constant elevation of the arm to a level corresponding to an arterial blood pressure reduction of 20 mm Hg did not alter adipose tissue blood flow At higher elevation autoregulation was not seen as blood flow fell in proportion to the reduction in perfusion pressure Below the heart where arterial and venous pressures in the forearm increase in parallel blood flow in subcutaneous tissue remained constant This indicates adjustments in arteriolar resistance to oppose the distending force of the increased transmural pressure In this orthostatic pressure range blood flow was maintained until a level corresponding to an increase in pressure of about 15 mm Hg beyond which blood flow was reduced to about 60 per cent

The ability of organs or tissues to keep their blood flow essentially constant during changes in arterial pressure is referred to as autoregulation The concept of a local myogenic response of vascular smooth muscle to change in transmural pressure was introduced by Bayliss (1902) suggesting the myogenic theory of autoregulation It has been questioned whether autoregulation is exclusively due to a myogenic response Thus the metabolic theory suggests anaerobic vasodilator metabolites liberated by the tissues as mediators of this physiological characteristic (Anrep 1912) Other theories have been proposed but the fundamental nature of the response is still unsettled (Rodbard 1971)

Autoregulation has previously been demonstrated in kidney (Rein 1931 Selkurt 1946) skeletal muscle (Roy and Brown 1880 Folkow 1949) liver (Torrance 1958) myocardium (Berne 1959) intestine (Burgi 1944 Johnson 1960) brain (Fog 1934 Rapela and Green 1964) and cutaneous tissue (Henriksen *et al* 1973)

Selective determination of adipose tissue blood flow is possible by the local xenon 133 washout technique Thus the purpose of the present study was to investigate the influence of orthostatic pressure changes on human adipose tissue blood

flow in the subcutaneous tissue of the distal part of the forearm in order to examine whether autoregulation occurs in this tissue.

Experimental procedure

The experiments were performed on 4 healthy subjects. 0.2—0.6 ml xenon 133 dissolved in isotonic saline (obtained from AB Atomenergi, Studsvik, Sweden) with an activity of 2.0—2.2 mCi/ml was injected intracutaneously in the right forearm 5 cm proximal to the wrist. This procedure was chosen in order to avoid trauma on the subcutaneous tissue. The subject was placed on a chair in a constant temperature room with an air temperature of 27 ±0.5 °C. Close to the deposit area a thermocouple was placed upon the skin. Temperature was read every 5 min to assure that the temperature remained within 0.5 °C from the initial value of each trial. *The right forearm was placed in an individually made plaster splint with the back* of the forearm uncovered. Care was taken to avoid compression, thus the splint was lined with 3 mm foam rubber. The splint was mounted on a horizontal bar allowing it to be positioned in any inclination. Horizontal and vertical dislocations were obtained by a counterbalance system.

A horizontal plane through the jugular notch was chosen as reference level.

A NaI (Tl) scintillation detector collimated to see more than the area of deposit was mounted 15—20 cm from the radioactive field. The pulses were fed into a universal printing gamma spectrometer (Meditronic, Denmark). The centerline was adjusted to the 0.081 MeV peak of gamma maximum energy of xenon 133, the window being symmetrical about this peak. Counting was started 95 min after the injection and the activity was printed out without time loss every 20th s. Count figures were in the range 6×10^4 to 1.2×10^6 per 20 s when the trials were initiated.

A complete trial consisted of three counting periods each on 6—10 min with the deposit area at first at the reference level, then displaced to the desired test level, and finally returned to the reference level. Several trials were carried out on the same deposit. A trial was accepted for further calculation if the washout curves obtained at the reference level were *estimated by eye to be parallel when plotted in a semilogarithmic diagram.*

Venous mean pressure $\bar{P}_v$ was determined for each subject by direct venopuncture. Arterial blood pressure was determined on the arm with the cuff 5 cm below a level through the jugular notch. Diastolic pressure was read when the sounds changed character (Roberts, Smiley and Manning 1953).

Calculations

Intracutaneous injection of xenon 133 elicits a hyperaemic trauma phase. Furthermore a two-compartmental distribution system is present in the washout of xenon 133 from cutaneous tissue. In about 60 min a monoexponential course of the washout curve is obtained indicating that the xenon 133 is now located exclusively in the subcutaneous tissue creating a final monocompartmental washout system (for details, see Sejrsen 1971). Intercompartmental diffusion processes between cutaneous and subcutaneous tissues for all practical purposes can be neglected *in the final monoexponential part of the washout curve, and recirculation of xenon 133 is in*significant (Sejrsen 1967, Tønnesen and Sejrsen 1967). Recently it has been shown that during steady state conditions the local xenon 133 washout method gives a reliable estimate of the blood flow in adipose tissue (Nielsen 1972).

Adipose tissue blood flow (f) can be calculated from the rearranged Kety equation $f = k \times \lambda \times 100$ (ml/100 g × min) where λ denotes the adipose tissue to blood partition coefficient for xenon 133 (Kety 1949). In the calculations a λ value of 10 ml/g was employed (Sejrsen 1971). The slope k of the regression line for the logarithmic transformed count figures against time was computed according to the method of least squares. Arterial mean pressure $\bar{P}$ (eff) was calculated by approximation, and from this the various $P_{a(i\,st)}$ values were computed.

For each trial it was statistically evaluated whether blood flow at the test level differed significantly from the corresponding two values at the reference level. If a u- or t-test respectively for the logarithmic blood flow values showed significant difference between the values of blood flow, autoregulation was regarded as absent. *If insignificance was shown,* autoregulation was considered present.

Results

Typical examples of the final part of the xenon 133 washout curve from two trials are shown in Fig. 1 a and 1 b. In Fig. 2 is presented the flow pressure relation in the

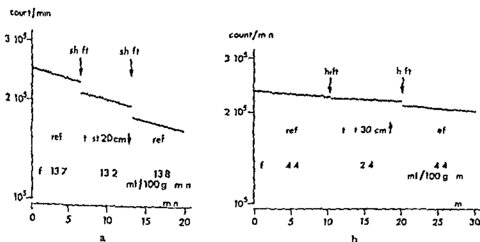


Fig 1 a and b illustrate typical xenon 133 washout curves obtained in two experiments. Fig 1 a shows autoregulation of blood flow in adipose tissue of the distal forearm when the investigated area is dislocated to a level 20 cm below the reference level the jugular notch (subject no 2) Fig 1 b demonstrates absence of autoregulation 30 cm above the reference level The blood flow decreases to 54 per cent of the value obtained at the reference level when the area is placed at a test level 30 cm above the reference level (subject no 3) Ref denotes reference levels f denotes adipose tissue blood flow

arterial pressure range where $\overline{P}_v$ is constant (subject no 2) The results are summarized in Table I Blood flow remained constant for all subjects in an interval around the reference level In extreme positions variation was observed Subject no 4 had no decrease of blood flow 40 cm above the reference level and subject no 3 had constant blood flow 40 cm below the jugular notch

The arterial and venous pressures are shown graphically together with the flow ratio and relative change in resistance at different test levels in Fig 3 The flow ratios remain constant from 20 cm above to 20 cm below the jugular notch Raising the

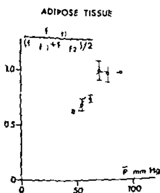


Fig 2 Flow pressure relation in the interval where venous pressure is constant i e from 5 cm below notch and upwards The horizontal bars

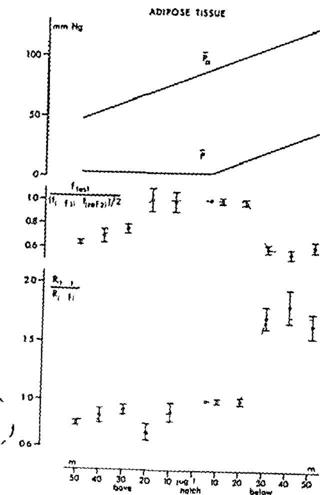


Fig 3 Diagram illustrating arterial mean pressure ($\overline{P}_a$) venous mean pressure ($\overline{P}_v$) fractional change of blood flow $\left(\frac{f_{(t\ t)}}{((f_{(ref\ 1)}+f_{(ref\ 2)})/2} \pm S E\right)$ and fractional change of resistance ($R_{(t\ st)}/R_{(ref)} \pm S E$) in various positions of the adipose tissue of the distal forearm when dislocated passively above and below the reference level the jugular notch Autoregulation of adipose tissue blood flow is seen to be present from 20 cm above to 20 cm below the reference level

arm above this interval produces a gradual decrease of the flow ratio A sudden decrease of the flow ratio was observed by lowering the arm to 30 cm below the reference level Further lowering to 40 cm and 50 cm below the reference does not decrease the flow ratio further As ($\overline{P}_a/\overline{P}_v$) is constant from 5 cm below the jugular notch and further down it follows that the ratio $R_{t\ t}/R_{r\ f}$) is constant = 1 in the interval from 5 cm to 20 cm below the jugular notch At 30 cm below the reference level the ratio of resistances is suddenly increasing and remains fairly constant as the arm is lowered further From 5 cm below to 20 cm above the jugular notch the ratio of resistances shows a linear decrease From 20 cm above the reference level and further up the ratio of resistances remains constant

TABLE I —denotes test levels above the jugular notch +denotes test levels below the jugular notch

Subject	Test level cm	No of Exp	$\frac{f_{(test)}}{(f_{(ref)} + f_{(\ )})/2}$	u or t	p
1	−30	4	0.8392	u = −4 725	p < 0 001
	−20	4	0 8912	u = −3 009	p < 0 01
	−10	6	0 9342	u = −2 963	p > 0 09
	+10	4	1 0118	u = 0 290	p > 0 7
	+20	6	1 0086	t = −0 017	p > 0 9
	+30	3	0 4527	t = −2 838	p > 0 1
n = 27 P̄ (n) = 85 mm Hg P̄ (n) = 3 mm Hg					
2	−50	3	0 6403	u = −7 105	p < 0 001
	−40	2	0 6983	u = −4 664	p < 0 001
	−30	3	0 7543	u = −3 227	p < 0 01
	−20	3	1 0920	u = 1 429	p > 0 1
	−10	3	0 9865	u = −0 255	p > 0 7
	+10	3	0 9959	t = 0 941	p > 0 4
	+20	2	0 9852	u = −0 190	p > 0 8
	+30	4	0 5909	u = −9 790	p < 0 001
	+40	2	0 5551	u = −7 679	p < 0 001
	+50	3	0 6141	u = −7 847	p < 0 001
n = 28 P̄ (en) = 90 mm Hg P̄v(n) = 3 mm Hg					
3	−40	3	0 7309	u = −7 424	p < 0 001
	−30	3	0 5643	u = −13 614	p < 0 001
	−20	3	0 8737	t = −1 538	p > 0 2
	+20	3	0 9285	u = −1 839	p > 0 05
	+30	4	0 8452	t = −2 131	p > 0 1
	+40	3	1 0136	u = 0 316	p > 0 7
n = 19 P̄ (en) = 95 mm Hg P̄ (n) = 4 mm Hg					
4	−40	2	0 6420	t − −1 045	p > 0 4
	−30	2	0 9785	u −0 267	p > 0 7
	−20	3	0 9784	u = −0 174	p > 0 8
	+20	4	0 9696	u = −0 246	p > 0 8
	+30	2	0 9178	u − −0 746	p > 0 4
	+40	2	0 7197	u − −2 634	p < 0 01
n₁ = 15 P̄ (n) = 90 mm Hg P̄ (n) = 4 mm Hg					

Discussion

Landis (1929) studied intravascular pressures in a capillary of the nail bed by a microinjection method Dislocating the finger below the jugular notch he observed an increase in pressure in the venous limb of almost the same magnitude as that produced by a column of blood of a height corresponding to the vertical dislocation The pressure in the arteriolar limb varied similarly from the reference level to about 30 cm below the reference whereafter the increase was less than on the venous side At this level a decrement in capillary pulsation was observed Above the reference level venous pressure remained constant and in the arteriolar limb pressure decreased less than expected from the theoretical hydrostatic pressure changes The perfusion pressure head is almost constant in the interval from the reference level to 30 cm below the heart for the capillaries and decreases outside this range in both directions Assuming resistance in the capillary to be constant capillary blood flow should be constant within the said interval and should decrease outside it

From 5 cm below the reference level and upwards venous mean pressure was found constant in the present study (Fig 3) and in this range a model similar to the *in situ* isolated and autoperfused pressure flow preparation was obtained (Folkow 1949)

The phenomenon of autoregulation is clearly shown in the interval from 20 cm above to 5 cm below the reference level R[illegible]/R[illegible] is constant in the interval from 30 cm to 50 cm above the reference level indicating that the compensatory vaso-relaxation has reached its maximum at 30 cm above the jugular notch and blood flow then decreases Assuming the apparent viscosity of the blood and the length of the vessels to be constant a change in resistance is due to a change in vascular caliber (Fig 2)

Transmural pressure increases when the arm is lowered and the volume of the various vessels should increase in proportion to their respective compliances provided the vessels behave passively From the present observations showing total resistance constant in this interval (Fig 3) it can be concluded that a compensatory vasoconstriction takes place

At 30 cm below the reference level and further down blood flow was decreased while the perfusion pressure head remained constant between capillaries and veins Thus an overcompensation of vasoconstriction takes place as R[illegible]/R[illegible] increases suddenly and remains constant downwards

The present observations are in accordance to deductions drawn from Landis experiments Coles and Greenfield (1956) found that blood flow in the hand did not change at a subatmospheric pressure of −30 mm Hg At −50 −100 and −150 mm Hg blood flow decreased Greenfield and Patterson (1954) had similar findings Roddie (1955) showed a tendency of increased blood flow by lowering down a finger 60 cm

It was only possible to evaluate the sum of resistances in the vascular bed As resistance probably decreased in capacitance vessels upon lowering the arm because of an increase of transmural pressure the compensatory increase of total resistance can be ascribed to vasoconstriction of the arterioles The mechanism underlying this vasoconstriction is unknown and this applies to the compensatory alterations observed at moderate lowering of the arm as well as to the overcompensation observed at extreme lowering As adipose tissue seems nearly deprived of influence from the baroreceptor system (Hanley Sachs and Skinner 1971) local reactions most likely are responsible It has been suggested that the mechanism should be a venous arteriolar reflex initiated by an increase in venous pressure (Haddy and Gilbert 1956) The arteriolar vasoconstriction will counteract an increase in capillary pressure and an ensuing increase in transcapillary filtration Teleologically the overcompensation can be visualized as a response counteracting edema formation at the expense of a diminution of tissue blood flow

It can be concluded that adipose tissue shows autoregulation of blood flow in the classical sense within a certain arterial blood pressure range where venous pressure is constant When arterial and venous pressures increase in parallel an arteriolar

muscle contraction takes place opposing the distending force of the increased trans mural pressure in order to keep resistance and thereby blood flow constant It is believed that autoregulation is due to local mechanisms but the mediators of this vascular response cannot be settled from this work

We wish to thank Dr Per Sejr en for valuable help and discussions

References

ANREP G On local vascular reactions and their interpretation *J Physiol* (Lond) 191? *45* 318—3?7

BAYLISS W M On the local reactions of the arterial wall to changes of internal pressure *J Physiol* (Lond) 1902 *28* 220—231

BERNE, R. M Cardiodynamics ard the coronary circulation in hypothermia *Ann N Y Acad Sci* 1959 *80* 365—383

BURGI S Zur Physiologie und Pharmakologie der uberlebenden Arterie *Helv Physiol Pharma col Acta* 1944 *2* 345—365

COLES D R. and A D M GREENFIELD The reactions of the blood vessels of the hand during increases in transmural pressure *J Physiol* (Lond) 1956 *131* 277—?89

FOG M *Om p aarteriernes vasomotoriske reaktioner* Thesis Copenhagen 1934

FOLKOW B *Intravascular pressure as a factor regulating the tone of the small vessels Acta physiol scand* 1949 *17* 289—310

GREENFIELD A D M and G C PATTERSON Reactions of the blood vessels of the human fore arm to increases in transmural pressure *J Physiol* (Lond) 1954 *125* 508—524

HADDY F J and R P GILBERT The relation of a venous arteriolar reflex to transmural pressure and resistance in small and large systemic vessels *Circulat Res* 1956 *4* 25—32

HANLEY H G R G SACHS and N S SKINNER Reflex respons veness of vascular bed of dog subcutaneous adipose ti sue *Amer J Physiol* 1971 *220* 993—999

HENRIKSEN O S L. NIELSEN W P PAASKE and P SEJRSEN Autorgulation of blood flow in human cutaneous tissue *Acta physiol scand* 1973 *89* 538—543

JOHNSON P C Autoregulation of intestinal blood flow *Amer J Phys ol* 1960 *199* 311—318

KETY S S Measurement of regional circulation by the local clearance of radioactive sodium *Am r Heart J* 1949 *38* 3?1—328

LANDIS E M Micro-injection studies of capillary blood pressure in human skin *Heart* 19?9 *15* ?09—228

NIELSEN S L Adipose tissue blood flow determined by the washout of locally injected ^{133}Xenon *Scand J clin Lab Invest* 1972 *9* 31—36

RAPELA C E and H D GREEN Autoregulation of canine cerebral blood flo *Ci culat Res* 1964 *15* Suppl I 205—211

REIN H Vasomotorische Regulationen *E gebn Phys ol* 1931 *32* 28—7?

ROBERTS L N J R SMILEY and G W MANNING A comparison of direct and indirect blood pressure determinations *Circulation* 1953 *8* 23? –242

RODBARD S Capillary control of blood flow and fluid exchange *Ci culat Res* 1971 *28* Suppl I 51—58

RODDIE R A Effect of arm position on circulation through the f ngers *J appl Physiol* 1955 *8* 67—7?

ROY C S and J G BROWN The blood pressure and its variations in the arterioles capillaries and smaller veins *J Physiol* (Lond) 1880 323—359

SEJRSEN P Diffusion processes in al dating the intraarterial Krypton 85beta pa ticle learance method for measurement of skin blood flow in man *C rculat Res* 1967 *1* ?81 –?95

SEJRSEN P *Measurement of cutaneous blo d flow by freely diffusible adioactive otop s* Thesis Copenhagen 1971 *Danish M dical Bull tin* 1971 Suppl 18

SELKURT E E *The relation of renal blood flow to effective arterial pressu e in the intact kidney of the dog Amer J Phys l* 1946 *147* 537—549

TONNESEN K. H and P SEJRSE Inert gas diffusion method for measurement of blood flow Comparison of bolus injection to directly measured blood flow in the isolated gastrocnemius muscle *Circulat Res* 1967 ?0 552—564

TORRA CE H B The control of the hepati arterial circulation *J roy Coll Surg Edi b* 1958 *4* 147

Acta physiol. scand. 1973. 89. 538—543

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Autoregulation of Blood Flow in Human Cutaneous Tissue

By

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Abstract

HENRIKSEN, O., S. L. NIELSEN, W. P. PAASKE and P. SEJRSEN. *Autoregulation of blood flow in human cutaneous tissue.* Acta physiol. scand. 1973. 89. 538—543.

The presence of autoregulation of blood flow was investigated in human cutaneous tissue of the skin fold between thumb and forefinger by means of the method of epicutaneous atraumatic application of gaseous xenon 133. 49 studies were performed on 3 healthy subjects. Blood pressure changes were obtained by passive displacement of the skin fold to various test levels above and below the reference level, the jugular notch. Blood flow remained constant for all subjects in an interval around the reference level. The range of autoregulatory ability showed variation. In extreme positions a decrease of blood flow was observed for some subjects. The significance of these findings is discussed.

During the last 25 years a growing interest has evolved concerning the phenomenon of autoregulation, i.e. the ability of tissues or organs to keep their blood flow essentially constant during changes in arterial pressure. Experiments performed on humans as well as on animals have disclosed the presence of autoregulation in kidney, skeletal muscle, liver, myocardium, intestine, brain and adipose tissue (Henriksen, Nielsen and Paaske 1973).

In the human forearm blood flow has been measured by means of venous occlusion plethysmography, by monitoring dissipation of heat, or by measuring arteriovenous oxygen differences. Changes in transmural pressure have been obtained by applying subatmospheric pressure to the forearm, or by means of orthostatic changes in the position of the extremity (Greenfield 1964, Coles and Greenfield 1956, Roddie 1955, Blair *et al.* 1959).

The advantages of experiments on animals are the possibility of complete control of pressure changes and exact blood flow determination. The obvious disadvantage is the operative trauma, involving a risk of disrupting the delicate mechanisms of autoregulatory response. In experiments on humans most procedures are atraumatic, but the blood flow detection is made by indirect methods and blood flow from the various tissues cannot be separated. Using the local washout technique of xenon 133 in subcutaneous adipose tissue it was possible to demonstrate autoregulation within

a certain range of orthostatic pressure changes (Henriksen *et al* 1973) By atraumatic epicutaneous labelling of the distal few mm of the skin fold between the thumb and forefinger with gaseous xenon 133 as introduced by Sejrsen (1967) it is possible to measure cutaneous blood flow selectively In the present study this technique has been used to examine whether autoregulation is present in human cutaneous tissue under normal physiological conditions

Experimental procedure

The experiments were performed on three healthy males By means of the epicutaneous atraumatic application of gaseous radioactive isotopes (Sejrsen 1967) the distal 3 to 4 mm of the skin fold between the thumb and forefinger of the right hand were labell d with gaseous xenon 133 (obtained from AB Atomenergi Studsvik Sweden) with a specific activity of 20 mCi/ml Diffusion processes were allowed to take place for 5 min The subject was then introduced to a constant temperature room with an air temperature of 21 ±0 5 C The subjects were normally dressed and had no subjective sensation of heat or cold The right hand was placed in an individually made plaster impression that was mounted on a horizontal bar allowing the plaster to be positioned in any inclination wanted The plaster could also be moved horizontally and vertically The position of the plaster could be changed easily and quickly using a counterbalance system to make the positional changes possible without effort A horizontal plane through the jugular notch was chosen as reference level The exact position of the deposit area in comparison to the reference level was determined with a spirit level

A NaI (Tl) scintillation detector collimated to see only the outer two mm of the skin fold was mounted on the horizontal bar and placed just above the radioactive f eld Thus the scintillation detector was constantly focusing the radioacti e area. The pulses from the scintillation detector were fed into a universal printing gamma spectrometer (Meditronic Denmark) The centerline was adjusted to the 0 081 MeV peak of gamma maximum energy of xenon 133 the window being symmetrical about this peak

Counting was started not later than 3 min after the termination of the gas labelling and the activity was printed out every 20th s without any time loss from the gamma spectrometer Count f gures were in the range 1500 to 5000 per 20 s when the experiments were initiated A complete trial consisted of three counting periods each of 3 to 6 min with the deposit area at first at the referer ce level then displaced to the desired test level and finally returned to the reference level Several trials were carried out on the same deposit. The count was corrected for background activity and plotted versus time during the experiments n a semilogarithmic diagram (base 10) A trial was accepted for further calculations if the lines obtained at the reference level were estimated by eye to be parallel

Calculations

After epicutaneous labelling with xenon 133 on the skin fold a monoexponenti l course of the washout curve is obtained (for deta ls and discus ion see Sejrsen 1971)

Rearranging Kety s original equation (Kety 1949) cutaneous blood flow f can be calculated by the equation $f = k \times \lambda \times 100$ (ml/100 g×min) where k is the rate constant of the washout curve and λ denotes the utaneous tissue to blood partit on coeff c nt for xenon 133 In the calculation a λ value of 0 7 n ml/g was used (Sejrsen 1971) A terial m an p essure at the test level and the relati change in resistance R(t t)/R(r) we e calculated as p e ented in anothe paper (Henriksen *et al* 1973)

Results

In Fig 1 is presented the flow pressure relation in the arterial pressure interval where venous pressure is constant (subject no 2) Blood flow is eemingly constant within the arterial pressure range from 90 to 70 7 mm Hg At lower arterial pressure blood flow seems to diminish

TABLE I —denotes test levels above the jugular notch +denotes test levels below the jugular notch

Subject	Test level cm	No of Exp	$\frac{f_{(test)}}{(f_{(ref1)}+f_{(ref2)})/2}$	u or t	p
1	−70	3	0 5317	u = −3 512	p < 0 001
	−50	3	0.5890	u = −2 866	p < 0 01
	−30	2	0 8357	u = −2 445	p > 0 01
	+30	2	0 9363	u = 0 067	p > 0 9
	+50	3	1 9699	t = 1 155	p > 0 3
	+60	2	0 9119	u = −0 380	p > 0 7
	+70	2	0 7362	u = −1 377	p > 0 1
	n_t = 17 $\overline{P}_{(ref)}$ = 85 mm Hg $\overline{P}_{(ref)}$ = 3 mm Hg				
2	−50	2	0 6981	u = −2 036	p > 0 0³
	−40	2	0 7549	u = −1 374	p > 0 1
	−30	2	0 9181	u = −0 269	p > 0 7
	−20	2	0 8494	u = −1 021	p 0 3
	+20	2	1 0746	u = 0 321	p > 0 7
	+30	2	1 1500	u = 0 999	p > 0 3
	+40	?	0 8344	u = −0 805	p 0 4
	+50	2	0 4889	u = −3 459	p < 0 001
	n = 16 $\overline{P}_{(ref)}$ = 90 mm Hg $\overline{P}_{(ref)}$ = 3 mm Hg				
3	−50	2	0 9765	t = −0 380	p 0 7
	−40	2	0 8133	u = −1 214	p 0 ?
	−30	2	0 9939	u = −0 032	p > 0 9
	−?0	2	0 8052	u = −1 112	p > 0
	+20	2	1 1340	u = 0 763	p 0 4
	+30	2	0 8047	u = −1 189	p 0
	+40	2	0 9615	u = 0 310	p 0
	+50	2	0 7976	u = −1 424	p 0 1
	n = 16 $\overline{P}_{(ref)}$ = 85 mm Hg $\overline{P}_{(ref)}$ = 2 mm Hg				

From Table I it is seen that blood flow remains constant except when the investigated area was placed in the extreme positions where blood flow was seen to decrease Interindividual variation was found however and subject no 1 showed only an insignificant decrease in blood flow at 70 cm below the reference level It is interesting to observe in subjects no 1 and 2 that blood flow decreases gradually as the arm is placed at levels from 30 cm above the reference level and upwards This finding corresponds to the results obtained for adipose tissue Subject no 2 shows insignificant fall in cutaneous blood flow at 50 cm above and 30 cm below the reference level In the interval between these two levels blood flow remains constant (Fig 2) The same subject showed autoregulation in the subcutaneous adipose tissue within the range from 30 cm above to 30 cm below the jugular notch It might be claimed therefore that cutaneous tissue of this subject has a better autoregulatory adjustment than subcutaneous adipose tissue Subject no 3 showed autoregulation at all investigated levels, from 50 cm above to 50 cm below the reference level

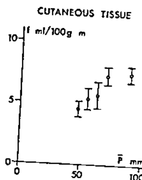


Fig 1 Flow pressure relation in the interval where venous pressure is constant i e from 5 cm below the jugular notch and upwards The horizontal bars signify S E

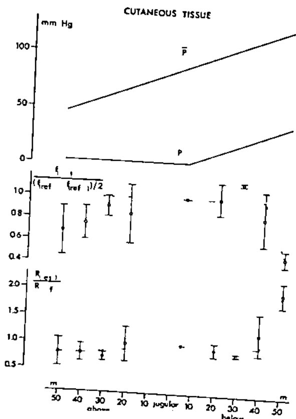


Fig 2 Diagram illustrating arterial mean pressure ($\overline{P}$) venous mean pressure ($\overline{P}_v$) fractional change of blood flow $\left(\frac{f_{(test)}}{(f_{(ref 1)} + f_{(ref 2)})/2} \pm S E\right)$ and fractional change of resistance ($R_{(test)}/R_{(ref)} \pm S E$) in various positions of the skin fold between the thumb and forefinger of the right hand when dislocated above and below the reference level the jugular notch Autoregulation of cutaneous blood flow is seen to be present from 40 cm above to 40 cm below the reference level The dotted line is drawn according to the results of the statistical evaluation cf Table I

Discussion

Autoregulation is clearly demonstrated in the interval from 5 cm below to 40 cm above the reference level in subjects no 2 and 3 In this range venous pressure remains constant but the perfusion pressure head decreases as the arterial mean pressure diminishes The finding of constant blood flow in this interval therefore implicates a compensatory decrement of resistance If vascular smooth muscle activity is constant below the jugular notch an increase in intravascular pressure implies an increase in intravascular diameter in proportion to the compliance of each vessel The fact that the perfusion pressure head remains constant together with a constancy of blood flow indicates an increase of the vascular smooth muscle activity in order to keep resistance constant The cutaneous vascular bed thus demonstrates an active response in almost the whole range of orthostatic pressure changes under physiological conditions In the upper extreme position the resistance cannot be reduced any further as the arteriolar muscular relaxation has reached its maximum in response to the decreasing perfusion pressure head At the lower extreme level an overcompensation is seen for subjects no 2 and 3 thus acting seemingly as an edema protecting factor by decreasing the high transmural capillary pressure in the fully dependent and relaxed arm The exact mediators of this response has not yet been established but a venous arteriolar reflex has been proposed (Haddy and Gilbert 1956) Deduction drawn from Landis experiments support the present findings (Landis 1929)

It is essential to point out that variation of the perfusion pressure head as determined by measurement of arterial and venous pressures does not necessarily correlate to changes in blood flow as the other important variable resistance might counteract changes in prefusion pressure head in vascular beds showing autoregulation as illustrated in Fig 2

A change in vasomotoric nervous activity might be anticipated during the trials However the experimental set up was chosen in order to minimize the effect of distorting factors

Coles and Greenfield (1956) found no changes in forearm blood flow when applying subatmospheric pressure at −30 mm Hg which corresponds to a position of the investigated area of 40 cm below the reference level At −50 −100 and −150 mm Hg blood flow decreased Greenfield and Patterson (1954) reported similar findings Roddie (1955) showed a tendency of increase in finger blood flow when lowering a finger to 60 cm below the jugular notch

In conclusion a compensatory change in resistance is present in cutaneous tissue in response to variations of intravascular pressure in order to keep blood flow constant and *per definitionem* autoregulation is present in this tissue

Autoregulation has up to now been demonstrated in many different tissues and organs Thus it seems reasonable to consider this physiological response a basic mechanism of local control of blood flow in the organism as a whole In the skin this local regulation can be influenced by central regulatory mechanisms i.e. blood pressure regulation and control of thermal homeostasis as the skin plays a central role in maintaining the internal thermal level of the body

An important question arises whether autoregulation is present in the vascular bed in patients suffering from occlusive arterial disease It is well known that patients suffering from intermittent claudication with resting pain get notable relief when the legs are dependent In our experiments however it was found that in normal subjects blood flow decreased in the cutaneous and in the subcutaneous tissues of the upper extremity in the extreme dependent positions It would be most interesting to examine the autoregulatory ability in patients with intermittent claudication especially in the distal part of the legs The local washout of xenon 133 does not appear to be a suitable method for investigating this subject in cutaneous tissue This is due to the biexponential washout system present in this area requiring 2 h of registration in steady state and a new depot for each measuring level

Measurements of cutaneous blood flow in regions other than the skin fold between the thumb and the forefinger must then await the development of other methods

References

BLAIR D A W E GLOVER A D M GREENFIELD and I C RODDIE The increase in tone in forearm resistance blood vessels exposed to increased transmural pressure *J Physiol* (Lond) 1959 *149* 614—625

COLES D R. and A D M GREENFIELD The reactions of the blood vessels of the hand during increases in transmural pressure *J Physiol* (Lond) 1956 *131* 277—289

GREENFIELD A D M Blood flow through the human forearm and digits as influenced by subatmospheric pressure and venous pressure *Circulat Res* 1964 *15* Suppl I 70—75

GREENFIELD A D M and G C PATTERSON Reactions of the blood vessels of the human forearm to increases in transmural pressure *J Physiol* (Lond) 1954 *125* 508—524

HADDY F J and R. P GILBERT The relation of a venous-arteriolar reflex to transmural pressure and resistance in small and large systemic vessels *Circulat Res* 1956 *4* 25—32

HENRIKSEN O S L NIELSEN and W P PAASKE Autoregulation of blood flow in human adipose tissue *Acta physiol scand* 1973 *89* 531—537

KETY S S Measurement of regional circulation by the local clearance of radioactive sodium *Amer Heart J* 1949 *38* 321—328

LANDIS E M Micro-injection studies of capillary blood pressure in human skin *Heart* 1929 *15* 209—228

RODDIE R A Effect of arm position on circulation through the fingers *J appl Physiol* 1955 *8* 67—72

SEJRSEN P Diffusion processes invalidating the intraarterial Krypton 85 beta particle clearance method for measurement of skin blood flow in vivo *Circulat Res* 1967 *21* 281—295

SEJRSEN P *Measurement of cutaneous blood flow by freely diffusible radioactive isotopes* Thesis Copenhagen 1971 *Danish Med cal Bull tin* 1971 Suppl 18

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Prostaglandin Mediated Inhibition of Noradrenaline Release at Different Nerve Impulse Frequencies

By

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Abstract

JUNSTAD M and Å WENNMALM *Prostaglandin mediated inhibition of noradrenaline release at different nerve impulse frequencies* Acta physiol scand 1973 89 544—549

The isolated perfused sympathetically innervated rabbit heart was stimulated for 6 min at 2 or 10 Hz and the outflow of noradrenaline was monitored by assay of the perfusate from the organ during nerve stimulation Continuous nerve stimulation caused a decline in the outflow of noradrenaline which was more pronounced at the higher nerve stimulation frequency When 5 8 11 14 eicosa tetraynoic acid a compound known to inhibit prostaglandin synthesis in this preparation was infused during nerve stimulation at 2 Hz the relative outflow of noradrenaline was increased by about 90 % while at 10 Hz no significant influence on the outflow of noradrenaline was observed The outflow of endogenous prostaglandins was slightly more than doubled when the stimulation frequency was increased from 2 to 10 Hz Simultaneously the sensitivity to exogenous prostaglandin E_2 of the chronotropic response to nerve stimulation was decreased The absence of endogenous inhibition of transmitter release at 10 Hz seems to be the result of both a decreased sensitivity of the noradrenaline release process to prostaglandins and an insufficient increase at this frequency in the amount of prostaglandin available to inhibit this process The experiments show that the prostaglandin mediated inhibition of the process of release of the sympathetic transmitter is more efficient at lower more physiological nerve impulse frequencies

The release of noradrenaline (NA) from adrenergic nerve terminals caused by impulses in sympathetic nerves can be depressed by exogenously administered prostaglandins (PG s) of the E series as has been shown in different tissues and species (for references see Hedqvist 1970 Wennmalm 1971) On the basis of these results it was suggested (Hedqvist 1969) that endogenous prostaglandins control the release of NA liberated during impulse activity in the sympathetic nerves Such an endogenous control possibly acting as a feed back system to restrict the release of NA was originally shown in the isolated perfused sympathetically stimulated rabbit heart (Samuelsson and Wennmalm 1971) In this preparation infusion of 5 8 11 14 eicosa tetraynoic acid a compound known to inhibit prostaglandin synthesis in vitro (Downing Ahern and Bachta 1970) was shown to cause a parallel inhibition

of PG synthesis and increase in release of NA. The results have been confirmed in other tissues (Hedqvist, Stjarne and Wennmalm 1971, Swedin 1971, Hedqvist 1972) and in the isolated rabbit heart, using another compound for inhibition of PG synthesis (Chanh, Junstad and Wennmalm 1972). Recently observations have been made which suggest that the endogenous PG "brake" on the release of NA acts not only in isolated tissues but also in intact animals (Stjarne 1971, Junstad and Wennmalm 1972).

In order to further characterize the function of the endogenous PG mediated inhibition of sympathetic transmitter release, its mechanism has been studied from different aspects more in detail in our laboratory. In the present paper the influence of the PG "brake" on the release of NA at two different impulse frequencies has been investigated.

Methods

Rabbits of mixed strains and sexes were used for the study. In one series they were killed by a blow on the head and bled from the left carotid artery. The heart with intact left and right sympathetic nerve supply was dissected out according to Huković and Muscholl (1962). The organ was perfused at a temperature of 37 °C and a pressure of about 60 cm H_2O with Tyrode's solution (conc. in mM: NaCl 136.9, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 1.0, $NaHCO_3$ 11.9, NaH_2PO_4 0.4, glucose 5.6) aerated with 6.5 % CO_2 in O_2. Ascorbic acid, 20 µg/ml, was added to the solution. The nerves with adjacent tissue were pulled through separate plastic tubes with platinum rings in their walls, serving as electrodes, and connected to Grass Model S 44 stimulators. They were stimulated by rectangular pulse trains of supramaximal strength and 1 ms duration. The apex of the heart was connected to a strain gauge transducer and heart rate and contractile force were recorded on a Grass Model 5 D Polygraph. The effluent from the heart was collected during the periods of nerve stimulation and until the contractile force had faded out. 5, 8, 11, 14-eicosatetraynoic acid (ETA), in ammonium form, was infused through a cannula close to the aorta to produce final concentrations of 10 M to 4×10^{-6} M.

Some hearts were used for studying the influence of ETA on the outflow of NA in response to nerve stimulation at different nerve stimulation frequencies. They were stimulated at 2 or 10 Hz. The effluent from the heart was collected in two min periods and immediately acidified and chilled. In some hearts ETA was infused from the beginning of the second 2 min effluent collection period and until the end of the stimulation. In the remaining hearts in this series solvent was infused and the experiments were performed similarly. The effluents were purified on alumina and assayed for NA according to Euler and Lishajko (1961). The ratio outflow of NA during the second or third 2 min period/outflow of NA during the first 2 min period was estimated both for hearts infused with ETA and for the controls. The difference between the ratio during the second period for hearts infused with ETA and the controls was statistically analyzed using Student's t test.

6 hearts were stimulated continuously for 6 min at 2 or 10 Hz and the effluent was assayed for PG using the extraction technique and bioassay on isolated rat stomach described earlier (Wennmalm and Stjarne 1971). In 6 other hearts the sensitivity of the nerve stimulation induced chronotropic response to exogenous PGE₂ was studied in the following way. The organ was stimulated at 2 and 10 Hz for 20 s at 10 min intervals for 3 times. 5 min prior to the second 2 and 10 Hz nerve stimulation PGE₂ (5×10^{-9} M) was infused through a cannula close to the aorta until the end of the nerve stimulation. The mean of the first and third 2 or 10 Hz chronotropic response (no PG infusion) was compared to the chronotropic response during the second nerve stimulation (performed during PG infusion).

Results

Stimulation of the sympathetic nerves to the heart at 2 or 10 Hz caused an increase in heart rate and contractile force, which was more pronounced at the h

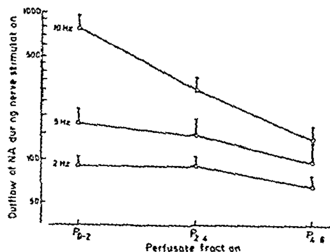


Fig 1 Outflow of NA during 3 consecutive 2 min periods of postganglionic sympathetic nerve stimulation at 2, 5 and 10 Hz. Data on 5 Hz from Samuelsson and Wennmalm 1971

stimulation frequency In addition there was an outflow of NA in the effluent at 2 Hz being 67 ± 9 (mean ± S E n = 12) ng during the first 2 min perfusate collection period and at 10 Hz 743 ± 88 (n = 10) ng During the following two 2 min perfusate collection periods the outflow of NA declined most markedly when the higher nerve stimulation frequency was used At 2 Hz the outflow during the second and third perfusate collection periods was 102 ± 2 (mean ± S E n = 6) % and 74 ± 12 (n = 6) % respectively of the outflow during the first perfusate collection period At 10 Hz the corresponding figures were 39 ± 6 (n = 5) and 18 ± 6 (n = 5) % of the outflow during the first period (Fig 1)

When ETA 10^{-6} M to 4×10^{-5} M was infused from the beginning of the second 2 min perfusate collection period the outflow of NA was markedly increased until the end of the nerve stimulation at 2 Hz while being unchanged at 10 Hz The outflow figures during infusion of ETA at 2 Hz were 191 ± 15 (n = 6) % and 99 ± 11 (n = 6) % during the second and third perfusate collection period The corresponding figures at 10 Hz were 41 ± 12 (n = 5) % and 12 ± 2 (n = 6) % of the outflow during the first perfusate collection period The increase in outflow of NA in the presence of ETA compared to controls at 2 Hz during the second perfusate collection period is statistically significant (P*** < 0 001) (Fig 2)

3 hearts were continuously stimulated at 2 Hz and the perfusate collected was extracted for PG Tested on an isolated rat stomach muscle strip the extract from the hearts all contained biological activity corresponding to 48 ± 10 (n = 3) ng PGE The perfusate from the 3 hearts stimulated at 10 Hz contained after a similar procedure of analysis biological activity corresponding to 113 ± 21 (n = 3) ng PGE_2

The sensitivity of the chronotropic response to exogenous PGE was tested at 2 and 10 Hz. At the lower frequency PGE_2 (5×10^{-7} M) inhibited the chronotropic response to 43 ± 9 (n = 6) % of the control response and at the higher frequency to

Fig 2 Outflow of NA during 3 consecutive 2 min periods of postganglionic sympathetic nerve stimulation at different frequencies expressed as percent of the outflow of NA during the first 2 min period Filled symbols prostaglandin synthesis inhibitor ETA infused from the beginning of P_{2-4} Open symbols solvent infused only Data on 5 Hz from Samuelsson and Wennmalm 1971

87±8 (n = 6) % The difference between the inhibition at 2 and 10 Hz is statistically significant (P** < 0 01)

Discussion

In the present study the outflow of NA during prolonged sympathetic nerve stimulation was studied in the presence and abscence and abscence of ETA a drug known to inhibit PG synthesis *in vitro* (Downing *et al* 1970) as well as *in vivo* (Samuelsson and Wennmalm 1971) At 2 Hz stimulation of the sympathetic nerves to the heart in the absence of ETA caused an overflow of NA which was even during the first part of the stimulation but declined somewhat towards the end At 10 Hz there was an initially very high overflow of NA which however declined rapidly and markedly The overflow of NA during the first 2 min perfusate collection period was more than 10 times higher at 10 Hz compared to 2 Hz Thus there was no arithmetic relationship between the increase in nerve impulse frequency and overflow of NA

Infusion of ETA from the beginning of the second perfusate collection period caused a significant increase in overflow of NA at 2 Hz while at 10 Hz no significant change in the outflow of NA from the heart was obtained The relative increase in overflow of NA observed at 2 Hz is in good accordance with earlier observations on facilitation of NA release followed by inhibition of PG synthesis in this organ (Samuelsson and Wennmalm 1971 Chanh Junstad and Wennmalm 1972) In these studies the increase in overflow of NA was caused by an increased release of transmitter which in turn was most probably caused by inhibition of the PG mediated restriction of the release of NA There is reason to assume that the same mechanism is responsible for the increase in overflow of NA observed at 2 Hz in the present study The failure by ETA to increase the overflow of NA in hearts stimulated at 10 Hz suggests that the PG mediated inhibition of transmitter release is not operating at this frequency The observation by Swedin (1971) that the faciliation by ETA of the mechanical response of the field stimulated guinea pig vas deferens was less pronounced at higher stimulation frequencies seems to point in the same direction Additional support for the view that the PG brake on NA release is abs[illegible] Hz is obtained by the observation that the initial outflow of NA was [illegible]

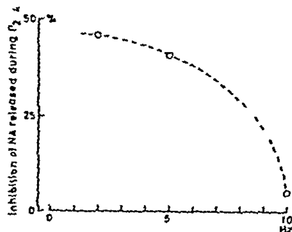


Fig 3 Influence of endogenous inhibition of the release of NA at different nerve impulse frequencies. Inhibition of NA release expressed as the relative difference between outflow of NA during the second 2-min perfusat collection period in hearts infused and not infused with prostaglandin synthesis inhibitor ETA Data on 5 Hz from Samuelsson and Wennmalm 1971

times higher at 10 Hz than at 2 Hz. Estimating the PG inhibition of NA release at 2 Hz to about 50 % gives a relation between the "uninhibited" outflow figures at the 2 frequencies mentioned which is more linear and thus fits better with the view that the amount of transmitter liberated per stimulus is constant (Folkow, Haeggendal and Lisander 1967).

The absence of endogenous inhibition of the release of transmitter at 10 Hz can theoretically be explained in terms of either a) lack of "sufficient" PG release at 10 Hz, b) decreased sensitivity for PG of the release process at 10 Hz or c) a combination of a and b. In the present study stimulation of the sympathetic nerves for 6 min at 2 Hz and 10 Hz caused an outflow of biologically active lipid corresponding to about 50 and 115 ng of PGE_2 respectively. By analogy with earlier studies in this organ (Samuelsson and Wennmalm 1971) it seems highly probable that the biological activity derives from the presence of PG s in the perfusate. The fact that the 5 times higher stimulation frequency only approximately doubled the outflow of PG s shows that the overflow of PG s is neither correlated in a linear way to the stimulation frequency nor to the amount of sympathetic transmitter released.

The chronotropic response of the heart to nerve stimulation in the present study was inhibited by exogenous PGE_2 more markedly at 2 than at 10 Hz. Thus the explanation for the lack of endogenous inhibition of the release of transmitter at 10 Hz seems to be dual in origin: when the nerve impulse frequency is raised there is an increase in the amount of PG available for inhibition of transmitter release, however, this increase is not capable to compensate for the decreased sensitivity of the release process for PG s.

In conclusion the endogenous PG mediated inhibition of sympathetic transmitter release operates at lower "physiological" nerve impulse frequencies while being uncapable to affect the release of NA at 10 Hz. The decrease in sensitivity to PG of the transmitter release process, more pronounced than the augmented amount of endogenous PG available is capable to compensate for, explains this failure of the endogenous PG "brake" at the higher nerve impulse frequency.

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References

CHANH P H M JUNSTAD and Å WENNMALM Augmented noradrenaline release following nerve stimulation after inhibition of prostaglandin synthesis with indomethacin *Acta physiol scand* 1972 *86* 563—567

DOWNING D T D G AHERN and M BACHTA Enzyme inhibition by acetylenic compounds *Biochem Biophys Res Commun* 1970 *40* 218—223

EULER U S v and F LISHAJKO Improved technique for the fluorimetric estimation of catecholamines *Acta physiol scand* 1961 *51* 348—355

FOLKOW B J HAGGENDAL and B LISANDER Extent of release and elimination of noradrenaline at peripheral adrenergic nerve terminals *Acta physiol scand* 1967 *72* Suppl 307

HEDQVIST P Antagonism between prostaglandin E2 and phenoxybenzamine on noradrenaline release from the cat spleen *Acta physiol scand* 1969 *76* 383—384

HEDQVIST P Studies on the effect of prostaglandins E1 and E2 on the sympathetic neuromuscular transmission in some animal tissues *Acta physiol scand* 1970 *79* Suppl 345

HEDQVIST P Prostaglandin mediated control of neurotransmission in guinea pig seminal vesicle *5th Int Congr Pharmac San Francisco 972* Abstract p 98

HEDQVIST P L STJARNE and Å WENNMALM Facilitation of sympathetic neurotransmission in the cat spleen after inhibition of prostaglandin synthesis *Acta physiol scand* 1971 *83* 430—432

HUKOVIC S and E MUSCHOLL Die Noradrenalin Abgabe aus dem isolierten Kaninchenherzen bei sympathischer Nervenreizung und ihre pharmakologische Beeinflussung *Naunyn-Schmiedeberg's Arch exp Path Pharmak* 1962 *244* 81—96

JUNSTAD M and Å WENNMALM Increased renal excretion of noradrenaline in rats after treatment with prostaglandin synthesis inhibitor indomethacin *Acta physiol scand* 1972 *85* 573—576

SAMUELSSON B and Å WENNMALM Increased nerve stimulation induced release of noradrenaline from the rabbit heart after inhibition of prostaglandin synthesis *Acta physiol scand* 1971 *83* 163—168

STJARNE L Hyperexcretion of catecholamines induced by indomethacine *Acta physiol scand* 1971 *83* 574—576

SWEDIN G Studies on neurotransmission mechanisms in the rat and guinea pig vas deferens *Acta physiol scand* 1971 *83* Suppl 369

WENNMALM Å Studies on mechanisms controlling the secretion of neurotransmitters in the rabbit heart *Acta physiol scand* 1971 *83* Suppl 365

WENNMALM Å and L STJARNE Inhibition of the release of adrenergic transmitter by a fatty acid in the perfusate from sympathetically stimulated rabbit heart *Life Sci* 1971 *10* 471—479

Acta physiol scand 1973 89 550—557

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Electrophysiological Study of the Gustatory Effects of the Sweet Proteins Monellin and Thaumatin in Monkey, Guinea Pig and Rat

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Abstract

BROUWER J N G HELLEKANT Y KASAHARA H VAN DER WEL and Y ZOTTERMAN *Electrophysiological study of the gustatory effects of the sweet proteins Monellin and Thaumatin in monkey guinea pig and rat* Acta physiol scand 1973 89 550—557

The afferent activity of the facial portion of the chorda lingual nerve the chorda tympani proper and the glossopharyngeal nerves was recorded during the application of 0 02 % and 0 005 % solutions of Monellin (2×10 and 5×10^{-6} M) and Thaumatin (10^{-5} and 5×10^{-6} M) and of 0 5 M sucrose 0 3 M NaCl and 0 03 M citric acid representing the three taste qualities sweet salt and acid Monellin and Thaumatin which give a sweet sensation in man elicited a significant electrophysiological response in the monkey which showed several characteristics that could be related to psychophysical observations in man In guinea pig and rat little or no response could be recorded

The number of proteins which act as taste stimuli or taste modifiers is growing Thus a taste modifying protein miraculin from the fruit of *Synsepalum dulcificum* has been characterized (Brouwer *et al* 1968 Kurihara and Beidler 1968) and used in psychophysical studies by Bartoshuk *et al* (1969) and Kurihara and Beidler (1968) and in an electrophysiological study by Diamant Hellekant and Zotterman (1972) Further a sweet tasting protein Thaumatin from the fruit of *Thaumatococcus daniellii* Benth was chemically characterized by van der Wel and Loeve (1972) Another sweet tasting protein Monellin from the fruit of *Dioscoreophyllum cumminsii* Diels was described by Morris and Cagan (1972) and van der Wel (1972) So far no electrophysiological study of the effects of Monellin and Thaumatin on the taste receptors recorded as the response in the taste nerves the chorda tympani proper and glossopharyngeal nerves has been reported In the present investigation the electrical response to these sweet tasting proteins in monkey guinea pig and rat was studied

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Methods

4 monkeys (*Cercopithecus aethiops*) 4 Sprague Dawley rats and 2 guinea pigs were used The monkeys were anesthetized with Mebumal® 30 mg/kg b wt The rats were anesthetized with Hypnorm® The initial dose of 0 5 ml/kg b wt was injected i m The analgesic was repeated when necessary with additional smaller doses (Hellekant 1971) The guinea pigs were anesthetized with urethane 1 5 g/kg b wt in a 40 % solution that was given intraperitoneally

After tracheotomy the right facial portion of the chorda lingual nerve the chorda tympani proper (Hellekant and Kasahara 1973) and the glossopharyngeal nerve were dissected The activity of the peripheral part of the cut nerves was recorded under mineral oil amplified and displayed on an oscilloscope from which it could be photographed The nerve activity was also recorded on a recorder Statos I (Varian assoc) as a summated trace Taste stimuli were applied with a device that has been described earlier (Andersson *et al* 1971) The solutions of Monellin and Thaumatin were applied for shorter time periods as a continuous flow and for longer either as intermittent flow or in cotton wool soaked in the solution in question Before the recordings were made from the glossopharyngeal nerve the right ramus of the mandible was cut to expose the posterior part of the tongue to the taste stimuli Solutions of 0 2 % 0 0? and 0 005 % Thaumatin (10^{-4} 10^{-6} and 2.5×10^{-6} M respectively) and 0 0? % and 0 005 % Monellin ($2\times10^{-?}$ and 5×10^{-6} M respectively) made up in tap water were used as test stimuli As standard stimuli 0 5 M sucrose 0 3 M sodium chloride and 0 03 M citric acid in tap water were used

Results

Recordings from the chorda tympani of the monkey

A series of summated responses to the standard stimuli and 0 02 % Thaumatin and Monellin in the monkey is shown in Fig 1 The records show that both test substances elicited a significant increase of activity in the chorda tympani The rise

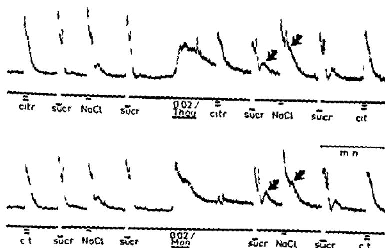


Fig 1 Summated activity in the chorda tympani nerve of a monkey recorded during stimulation with 0 03 M citric acid (citr) 0 5 M sucrose (suc) 0 3 M sodium chloride (NaCl) 0 0? Thaumatin (Thau) and 0 0? Monellin (Mon) Each stimulus was followed by a continuous flow of water at the same temperature over the tongue The signal below the recordings indicate the stimulation

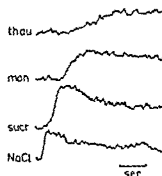


Fig 2 Summated activity of the chorda tympani nerve of a monkey recorded during stimulation with 0 02 % Thaumatin 0 02 % Monellin 0 5 M sucrose and 0 3 M sodium chloride Recording was started at the onset of stimulation

time of this increase was slower than that to any of the standard stimuli used This fact is further demonstrated in Fig 2 It is also apparent from this figure that the latency between onset of flow and response for both Thaumatin and Monellin is longer than that for sucrose and sodium chloride

As shown in Fig 1 the maximum activity obtained with the sweet proteins was lower than that of the standard solutions Table I summarizes the results obtained The magnitude of the responses to Monellin and Thaumatin in each animal was expressed as a percentage of their responses to sucrose From the magnitude of these responses it can be calculated that on a molar basis Thaumatin is for the monkey between 27 000 and 81 000 times as sweet as sucrose and Monellin between 19 000 and 50 000 times which is largely in agreement with psychophysical data obtained in man (van der Wel 1972 van der Wel and Loeve 1972)

Another remarkable feature of the pattern of response to the sweet proteins is the slowness with which the summated trace returns after stimulation to the level observed before stimulation In the upper trace of Fig 1 an increase of activity can still be observed 2 min after the application of Thaumatin and Monellin though the tongue had been rinsed with water and other taste solutions In addition it can be seen that the responses to sucrose following Thaumatin or Monellin were depressed The responses to citric acid and sodium chloride were not affected

Following the exposure to the sweet proteins an after response was observed

TABLE I Response of the sweet proteins relative to sucrose The responses to Thaumatin and Monellin were expressed in per cent of the response to 0 5 M sucrose Then the average value $\bar{x}$ was calculated The number of determinations is indicated by the figure n to the right of each $\bar{x}$

Thaumatin		Monellin	
10^{-5} M	2 5 × 10 M	2 × 10 M	5 × 10^{-4} M
$\bar{x}$ n	$\bar{x}$ n	$\bar{x}$ n	$\bar{x}$ n
54 11	43 7	66 11	45 6

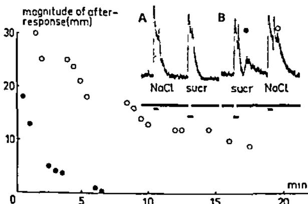


Fig 3 Magnitude of the after response to NaCl (O) and sucrose (●) plotted against the time elapsed after application of Thaumatin A and B are examples of the responses before and after Thaumatin the after responses to NaCl being indicated by the symbol O and that to sucrose by ●

during the water rinse subsequent to stimulations with sucrose or sodium chloride solutions This can be seen in Fig 1 where some to the after responses are indicated by arrows (The after responses which can be seen at the NaCl responses before the Thaumatin and Monellin in Fig 1 emanate from earlier application of these two substances) It should be noted that we consider the peaks appearing 3 s after a salt stimulus or 8 s after a sucrose stimulus as the after responses and not the short peak appearing at the moment the stimulus is replaced by the water rinse The after response to salt was more evident lasted longer and came sooner after the stimulus response than that after sucrose Fig 3 gives a plot of the after response against time as well as an illustration of the shape of the responses to salt and sucrose before and after Thaumatin No after response was observed after citric acid or when an intermittent flow of water was used

Recordings from the glossopharyngeal nerve of the monkey

The response to 0 02 % Monellin and Thaumatin were approximately one half of that to sucrose An example is shown in Fig 4 which shows the response to sucrose 0 02 % Monellin and 0 02 % Thaumatin The responses to the lower concentrations of the test stimuli not shown here were smaller but still significant The response to sucrose was depressed after the application of the test solutions The rate of increase in nerve activity during stimulation with Monellin and Thaumatin was in a similar way as for the chorda tympani lower than that caused by the standard solutions

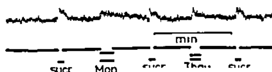


Fig 4 Summated activity in the glossopharyngeal nerve of a with 0 5 M sucros, 0 0?

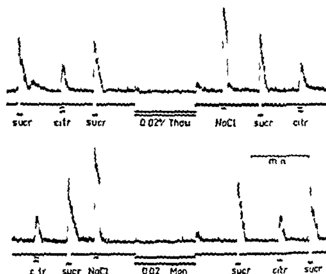


Fig 5 Summated activity in the chorda tympani nerve of a guinea pig

Observations made in the guinea pig

The response of the chorda tympani to sucrose in the guinea pig is relatively large Thaumatin and Monellin were therefore tested in this species However neither of hese substances which were applied at a concentration of 0 02 % gave a significant onse in the chorda tympani nerve Fig 5 shows the results of such an application cotton wool soaked in the stimulus in question No noticeable increase of activity observed The increase of activity which can be observed after the stimulation was the result of mechanical and thermal stimulation caused by the subsequent water rinse It was not observed when the method of rinsing the tongue was used The effects of the standard solutions show that the absence of a significant response did not depend on the recording conditions It can also be observed that the test substances did not depress the subsequent responses to sucrose as they did in the monkey Recording of the glossopharyngeal nerve gave similar negative results

Observations made in the rat

Preliminary behavioural experiments by one of us (van der Wel) demonstrated that rats prefer 0 001 % Thaumatin to water in a two bottle test Therefore it was of interest to record from the chorda tympani and the glossopharyngeal nerves in the rat during the application of this substance to its tongue

However neither Thaumatin nor Monellin caused any apparent increase in the activity of these nerves Fig 6 is recorded from the chorda tympani nerve to stimulation of the tongue with 0 02 % Thaumatin upper trace and 0 02 % Monellin lower trace A slight increase is seen during the stimulation with Thaumatin But this increase is the result of cooling of the tongue because continuous flows could not

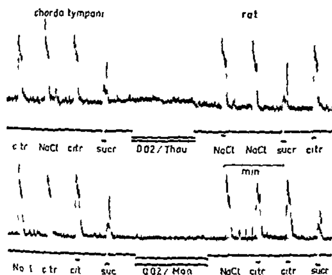


Fig. 6. Summated activity in the chorda tympani nerve of a rat.

be used. Nevertheless, it can be concluded from this and other records that no detectable response was elicited by Monellin and Thaumatin.

A 0.2% solution of Thaumatin was tested in one animal. It elicited some increase in the chorda tympani activity. This activity was not caused by the method of applying the stimulus to the tongue because when water was applied to the tongue in a similar intermittent way there was no increase in activity.

Recordings from the glossopharyngeal nerve gave similar results. Fig. 7 shows an example of this. It can be observed that the sucrose solution gave a response while the 0.02% Thaumatin solution gave no increase of activity but rather a small decrease.

Discussion

The results of the present study show that in the three species tested Monellin and Thaumatin elicit a significant response only in the taste nerves of the monkey. In man Monellin and Thaumatin elicit a sweet taste with a sweetness intensity relative to sucrose of 68 000 and 100 000 respectively (on a molar basis). The results of this study indicate that for the monkey these intensities are comparable. (Average values of 34 000 and 34 000 respectively.) In man cross-adaptation with sucrose and blocking of the sweetness by gymnemic acid seem to point to the sweet proteins having



Fig. 7. Summated activity in the glossopharyngeal nerve of a rat.

the same mechanism of sweet stimulation as sucrose (van der Wel 1972) From our observations cross adaptation with sucrose was apparent in the monkey as well the response to sucrose was depressed by previous application of the test substances This may indicate that Monellin and Thaumatin compete with sucrose for the receptor sites (Hellekant 1969) Further the typical long time lag and slow rise time (Fig 2) of the response parallels the slow development of the sweet sensation man experiences when tasting these proteins Finally the after response following salt stimulation (Fig 1 and 3) indicates a taste sensation which may be comparable to the sweet taste of water after Thaumatin reported earlier for man (van der Wel 1972) This taste can be repeatedly experienced when the mouth is rinsed with water after a previous application of Thaumatin saliva possibly serving as the salt solution in this case Thus from the observations discussed here it can be concluded that our findings in the monkey parallel in many respects the preceptual observations in man

In guinea pig and rat practically no response was elicited from the taste nerves by Monellin and Thaumatin This absence of a response is not related to the fact that we had to use cotton wool soaked in the stimulus for the one min stimulation A continuous flow would have given the same result This is supported by the fact that the response to alcohol which is built up very slowly in the rat (Hellekant 1965) shows the same rate of increase when the alcohol is applied in cotton wool as a continuous flow The reason why a continuous flow is preferred is that no effect of cooling the tongue or of mechanical stimulation will obscure the results when the stimulus is removed Further we tried a continuous flow for 20 s in a few cases and [illegible]ained the same results

Our experiments show that rats are able to taste Thaumatin even at low concen[illegible]ations as is apparent from their preference for 5×10^{-7} M (0 001 %) Thaumatin to water in a behavioural test In analogy with this is the observation that rats discriminate between 1 5 M alcohol and water though 1 5 M alcohol gives no increase or a slight depression of the summated recording from the chorda tympani proper nerve (Hellekant 1965) These and earlier observations show that between two different taste stimuli the magnitude of the electrophysiological response can not be used as a measure of the taste sensation evoked while for the same stimulus there is a good correlation between the magnitude of the electrophysiological response and the strength of the taste sensation (Borg *et al* 1967) Thus the fact that Monellin and Thaumatin evoked no or little change in the summated nerve activity of the chorda typmani or the glossopharyngeal nerves does not mean that these substances do not taste to the rat or the guinea pig

It is known that the amount of cross adaptation between two taste stimuli serves as a good measure of the similarity of the taste sensations they elicit (Frank *et al* 1972) The absence of any cross adaptation between Monellin or Thaumatin and sucrose in the guinea pig and the rat (Fig 5 and 6) indicates that these substances do not elicit a sensation of the same type as sucrose does This is supported by the finding of one of us (van der Wel) that in a choice between 1 % sucrose and 0 001 % Thaumatin rats preferred sucrose

In summary this discussion suggests that Monellin and Thaumatin to the monkey have a taste which is similar to that of sucrose and that they taste different to the rat and the guinea pig

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References

Andersson I G Hellekant R Larsson C Molander and L Ström A system for manually or automatically controlled gustatory stimulation *Med biol Engin* 1971 *9* 715—717

Bartoshuk L M G P Dateo D J Vandenbelt R L Buttrick and L Long Jr Effects of Gymnema sylvestre and Synsepalum dulcificum on taste in man *Olfaction and Taste III* pp 436—444

Borg G H Diamant L Strom and Y Zotterman The relation between neural and perceptual intensity A comparative study on the neural and psychophysical response to taste stimuli *J Physiol* (Lond) 1967 *192* 13—20

Brouwer, J N H van der Wel A Francke and G J Henning Miraculin the sweet inducing protein from Miracle fruit. *Nature* (Lond) 1968 *220* 373—374

Diamant H G Hellekant and Y Zotterman The effect of Miraculin on the taste buds of man monkey and rat *Olfaction and Taste IV* 1972 pp 241—244

Frank M D V Smith and C Pfaffmann Cross-adaptation between salts in the rat s chorda tympani response *Oral Physiology* 1972 pp 227—237

Hellekant G Electrophysiological investigation of the gustatory effect of ethyl alcohol The summated response in the chorda tympani in the cat dog and rat *Acta physiol scand* 1965 *64* 392—397

Hellekant G Inhibitory processes in gustation *Acta physiol scand* 1969 *75* 39—48

Hellekant G Efferent impulses in the chorda tympani nerve of the rat *Acta physiol scand* 1971 *83* 203—209

Hellekant G and Y Kasahara Secretory fibres in the trigeminal part of the lingual nerve to the mandibular gland of the at *Acta physiol scand* 1973 *89* 198—207

Kurihara K and L M Beidler Taste modifying protein from Miracle fruit *Science* 1968 *161* 1241—1243

Kurihara, K and L M Beidler Mechanism of the action of taste modifying protein *Nature* (Lond) 1969 *22* 1176—1179

Morris J A and R H Cagan Purification of Monellin the sweet principle of Dioscoreophyllum cumminsii *Biochim biophys Acta* (Amst) 1972 *261* 114—122

Wel H van der Isolation and characterization of the sweet principle from Dioscoreophyllum Cumminsii (Stapf) Diels *FEBS Letters* 1972 *21* No 1 88—90

Wel H van der and K Loeve Isolation and characterization of Thaumatin I and II the sweet tasting proteins from Thaumatococcus daniellii Benth *Eur J Biochem* 1972 *31* 221—225

Acta physiol scand 1973 89 558—570

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Cardiovascular Effects of Blood Borne Material Released from the Cat Small Intestine during Simulated Shock Conditions

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Abstract

HAGLUND U and O LUNDGREN *Cardiovascular effects of blood borne material released from the cat small intestine during simulated shock conditions* Acta physiol scand 1973 89 558—570

Cardiac performance was studied in the cat during and after a mimicked shock situation in he small intestine (regional hypotension at 35 mm Hg during nervous vasoconstrictor fibre tivation) Aortic blood flow was measured with an electromagnetic flow probe around the scending aorta and in some experiments maximal dP/dt of the left ventricle was also llowed Upon restoration of intestinal perfusion pressure after a 2 h shock period a rapid eduction of aortic flow occurred due to a decreased stroke volume Concomitantly maximal dP/dt was reduced in the face of an unchanged central venous pressure and left end diastolic pressure This decline of cardiac performance could be prevented by substituting during the first posthypotensive min the intestinal venous blood with fresh blood from a healthy donor animal However when the shed intestinal venous blood was retransfused a rapid decline of cardiac performance was evident The reduced cardiac function was accompanied by an unchanged or slightly elevated total peripheral resistance It is proposed that substances released from the shocked intestine cause a rapid and pronounced depression of cardiac function

The role of a reduced intestinal blood flow in the development of shock has been intensely discussed since Lillehei proposed the existence of an intestinal factor in irreversible hemorrhagic shock (Lillehei 1957) As reviewed by Selkurt (1970) many different vasoactive substances have been proposed to be released from the splanchnic area during or after periods of reduced blood flow Among these are a myocardial depressant factor suggested to be a polypeptide released from the pancreas and claimed to be important for the development of irreversible shock (Lefer 1970) Furthermore Fine (Fine *et al* 1959 Fine 1967) has suggested that absorption of endotoxins across a damaged mucosa should be of crucial importance while others have failed to confirm this view (*e g* Carter and Einheber 1966) or favoured more multifactorial pathogenesis (*e g* Kuida 1967)

In previous experiments (Haglund and Lundgren 1972 a b Ahren and Haglund 1973) in which the hemodynamic effects of regional hypotension on the cat small intestine were studied a pronounced cardiovascular derangement was noticed once the perfusion pressure was restored as reflected by a continuously falling blood pressure A similar chain of events was also observed when the effects of general hemorrhagic hypotension were studied (Haglund and Lundgren 1973 Haglund 1973) These cardiovascular effects cannot be attributed to any pancreatic release of a myocardial depressant factor since virtually the whole pancreas had been extirpated and the minor remnant was never exposed to hypotension in the first mentioned series of experiments (Haglund and Lundgren 1972 a b) Furthermore the mentioned cardiovascular derangement could not be ascribed to endotoxins The deleterious acute effects of this substance are due to pulmonary vasoconstriction greatly increasing right atrial pressure (Kuida *et al* 1961 Greenway and Murthy 1971) but such pressure rises were never observed in the earlier studies (Haglund and Lundgren 1972 a b 1973)

The aim of the present study was twofold First the experiments were so arranged as to obtain further evidence that a period of reduced intestinal blood flow leads to a release of substances from the gut which can cause a general cardiovascular deterioration Second it was considered to be of interest to elucidate if this deterioration was primarily due to cardiac failure or a matter of vascular derangement

Methods

A Operative procedures and recording of cardiovascular parameters Experiments were performed on 17 cats weighing 2 6—4 5 kg anesthetized i v with chloralose (50 mg/kg b w) after induction with ether The cats had been deprived of food for 12 h and had no obvious signs of intestinal infection

The abdomen was opened in the midline and an intestinal segment weighing 50—70 g was isolated while the remainder of the intestine as well as the spleen the greater omentum and a major part of the pancreas were extirpated The superior mesenteric artery being the only supplying vessel to the isolated intestinal segment was dissected free close to the aorta and an adjustable clamp was placed around this vessel making it possible to lower the intestinal arterial inflow pressure to any desired level (for details see Haglund and Lundgren 1972 a) The left adrenal gland was denervated and the vessels supplying the right one were ligated The nerves surrounding the superior mesenteric artery were cut and the distal end was placed on a ring electrode for electrical stimulation (6 Hz, 6 ms 12 V) with a Grass stimulator Atropine (1 mg/kg b w) was given i

The thorax was opened by a left parasternal transcostal incision In order to sympathetically denervate the heart all connections between the heart and the stellate ganglia were cut bilaterally An electromagnetic flow probe (Micron Instruments Inc Los Angeles Calforn USA) was placed around the ascending aorta to record cardiac output minus coronary flow The flow probe was coupled to a Medicon Microflow® electromagnetic flowmeter (Model 2000 Medicon Los Angeles California, USA)

After heparinization the left femoral artery and a minor branch of the superior mesenteric artery distal to the adjustable clamp were cannulated for blood pressure recordings by means of a pressure transducer (Statham P23) and a mercury manometer respectively The venous outflow from the intestinal segment and its lymph nodes was recorded continuously by an optical drop recorder unit venous outflow pressure being set to 10 mm Hg The was via a funnel returned to the animal through a catheter in a jugular vein w

placed just close to the right atrium thus making it possible to estimate indirectly changes in central venous pressure (CVP)

In 5 of the animals a thin catheter connected to a pressure transducer (Statham P23G) was placed in the left ventricle *via* the left heart auricle The peak rate of change of the left ventricular pressure during the isometric contraction phase (maximal dP/dt Linden 1968) was continuously recorded by means of an electronic derivator device (constructed by civ ing Lars Stage in this department) coupled to the pressure transducer

In three control experiments regional hypotension was instead induced in a denervated skeletal muscle preparation The adjustable clamp was then placed around the external iliac artery and the deep femoral artery cannulated for arterial inflow pressure recordings Hind limb flow was measured with a drop recorder unit connected to the femoral vein The hind limb was skinned and the paw was ligated so that the recorder measured mainly muscle blood flow Also in these experiments the discharge from the adrenal glands was eliminated The intrathoracic operative procedures were identical to those described above

B Acid base balance of the experimental animal During the whole experiment a slow i v infusion of a bicarbonate solution (10 meq $NaHCO_3$ per 100 ml 10 % glucose solution 0 10—0 15 ml/min) was given to reduce the effects of the surgical trauma on the acid base balance (Haglund and Lundgren 1972 a b) Just prior to the thoracotomy and throughout the experiment the cats were artificially ventilated To avoid atelectasis the tidal volume was set at 15 ml/kg b w i e slightly above normal at a frequency of 15/min A gas mixture containing 97 % O_2 and 3 % CO_2 was used This procedure has proved to keep arterial P_{CO_2} close to the normal value in similar experiments (Thoren personal communication)

C Experimental procedures After the operative procedures the animals were allowed to rest for 30 min Then one of three types of experiments was performed

1 Arterial inflow pressure to the small intestine was lowered to about 35 mm Hg for 2 h during continuous electrical stimulation of the regional vasoconstrictor fibres at 6 Hz The animals were followed for 1 h after the hypotensive period (9 animals)

2 A 2 h period of regional hypotension and constrictor fibre stimulation was performed as described above but the intestinal venous outflow was collected in a plastic beaker for about 5 min immediately after the hypotension Instead fresh blood from another cat was given i v in equivalent amounts i e an exchange transfusion was performed (below named exchange transfusion I) 45 min later the shed intestinal venous blood which had been stored in a refrigerator was rewarmed to body temperature and given intravenously to the experimental animal while an equivalent amount of arterial blood was withdrawn comcomitant (exchange transfusion II) The circulatory parameters were followed for another hour after the second exchange transfusion (5 animals)

3 A 2 h period of regional hypotension in the hind limb was performed as described above for the intestine except that the regional vasoconstrictor fibres were not stimulated Instead inflow pressure was lowered to such a level as to induce a flow reduction of the same magnitude as seen initially in the intestinal experiments i e to about 1/3 of control

To minimize rheological disturbances 2 ml of a dextran solution (1/3 Rheomacrodex® and 2/3 Macrodex® Pharmacia Sweden) was given every 20 min in all experiments Furthermore the drop chamber the funnel and the tubing system were primed with this solution before connection to the animals Thus the total amount of dextran given was of the same order of magnitude as had been shown to reverse rheological disturbances in dog skeletal muscle during shock (Appelgren and Lewis 1972)

D Calculations Regional perfusion pressure regional blood flow systemic arterial (or systolic) pressure flow in the ascending aorta and in some experiments maximal dP/dt of the left ventricle were recorded continuously Heart rate was determined from the aortic flow curve The base line of the electromagnetic flow recorder was easily checked since blood flow in the ascending aorta is zero during part of the ventricular diastole Such a check was made at regular intervals throughout each experiment otherwise mean aortic flow was registered The electromagnetic flowmeter was calibrated with saline after each experiment with the probe *in situ*

From these cardiovascular parameters the following calculations were made Regional and total peripheral resistance were calculated from pressure and flow Stroke volume could be estimated from aortic flow and heart rate External cardiac work (CW) was calculated from the formula

$$CW = \text{stroke volume (ml)} \times \text{mean aortic pressure (m } H_2O)$$

disregarding the kinetic energy imparted to the blood which only amounts to 5 per cent of total CW (*cf* Folkow and Neil 1971) CW was expressed in ergs

Regression lines were calculated by the method of least squares Statistical significance ($p < 0.05$) was calculated by the Wilcoxon matched pair signed rank test or when the material was too small ($n = 5$) the sign test (Siegel 1956)

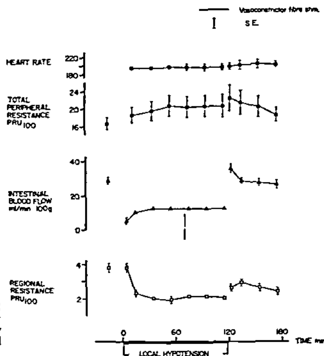


Fig 1 The effects of a mimicked shock situation in the intestine (prolonged intestinal hypotension with stimulation of the vasoconstrictor nerves) on heart rate total peripheral resistance intestinal blood flow and regional intestinal flow resistance (n = 9 for total peripheral resistance n = 7)

Results

A Intestinal experiments In 9 animals a local intestinal hypotension at a pressure around 35 mm Hg was induced combined with stimulation of the regional vasoconstrictor fibres During the posthypotensive control period two of the animals died. Fig 1 illustrates the changes of heart rate total peripheral resistance (TPR) intestinal blood flow and intestinal flow resistance in these animals Heart rate stayed virtually constant throughout the experiments while TPR rose above control during the hypotensive period as well as after restoring regional perfusion pressure The slight fall in TPR late in the posthypotensive period was caused by a final vasodilatation recorded in the two dying animals If these two animals are excluded from the material TPR stayed largely constant in the posthypotensive period It is further evident that intestinal blood flow levelled off at approximately 50 per cent of the control value after an initial pronounced decrease Regional flow resistance on the other hand was first unchanged during the hypotensive period but after 10 min it fell to about 50 per cent of control and remained that low throughout the hypotension, despite the continuous vasoconstrictor fibre stimulation (black bar on the abscissa) The initial transient vasoconstriction during the hypotension probably represents the characteristic blood flow response induced by the vasoconstrictor fibres also seen at a normal perfusion pressure Upon releasing the partial occlusion intestinal blood flow first exceeded the control level but then it re-

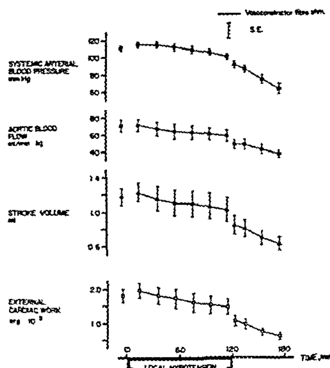


Fig 2 Changes in arterial blood pressure aortic blood flow stroke volume and external cardiac work during and after local intestinal hypotension with stimulation of the vasoconstrictor nerves (n = 7)

gradually to the control level while resistance approach the level observed during hypotension

Aortic blood flow was recorded in 7 of the animals of Fig 1 thus allowing estimations of stroke volume and external cardiac work if the coronary flow fraction is disregarded (Methods section D) These parameters as well as systemic arterial blood pressure and aortic blood flow are illustrated in Fig 2 It is seen that arterial pressure after a small initial increase showed a slow continuous fall during the hypotension period This pressure decline was much accelerated after releasing the adjustable clamp around the superior mesenteric artery A similar response pattern was also recorded for aortic flow calculated stroke volume and external cardiac work. The fast decrease of these vascular parameters observed in the very early part

TABLE I Maximal dP/dt (mm Hg/s) before during and after a 2 h period of local intestinal arterial hypotension. During the hypotension the regional vasoconstrictor fibres were stimulated at 6 Hz Mean value ± S.E. (n = 5)

	Control	Hypotensive period			Posthypotensive period		
Time min		35	75	115	3	35	55
Max dP/dt mm Hg/s	4300 ± 700	4300 ± 600	3600 ± 300	3500 ± 300	2900 ± 250	2700 ± 200	2200 ± 200

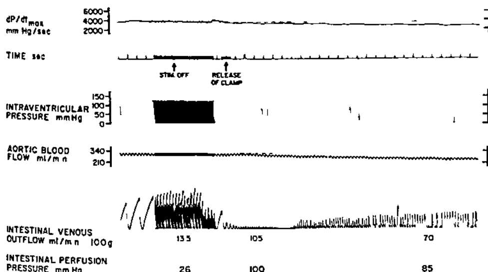


Fig 3 Cat 4 0 kg The acute effects of ending the vasoconstrictor fibre stimulation (stim off) and restoring the intestinal perfusion pressure (release of clamp) on dP/dt_m intra ventricular pressure aortic blood flow and intestinal blood flow after a 2 h intestinal "shock period Intraventricular pressure was measured with a catheter inserted *via* the left atrium

of posthypotension was statistically significant already within 1—5 min after restora tion of regional perfusion pressure Central venous pressure as indirectly recorded *via* the jugular vein was during this period if anything increased 1—3 cm H O above the control level of 2—5 cm H O

In 5 animals maximal dP/dt was recorded as described in Methods As is evident from Table I maximal dP/dt responded qualitatively as *e g* stroke volume and ex ternal cardiac work (*cf* Fig 2) In order to measure left ventricular end diastolic pressure more accurately than with the plastic tube inserted through the left atrium two experiments were performed in which maximal dP/dt was recorded *via* a rigid metal cannula inserted through the ventricular wall at the apex into the left ventricle The decrease of maximal dP/dt registered when administering venous plasma from a shocked intestine of another cat was accompanied with an unchanged or slightly increased end diastolic pressure

In order to illustrate further the pronounced cardiovascular changes seen upon releasing the partial occlusion of the superior mesenteric artery an experimental recording of left ventricular pressure maximal dP/dt aortic flow and total intestinal blood flow are shown in Fig 3 Within 30 s after the release of the partial vascular occlusion a marked decrease of maximal dP/dt and aortic flow combined with in creased CVP was recorded while heart rate was unaltered throughout the illus period Such reductions in cardiac performance were never seen in

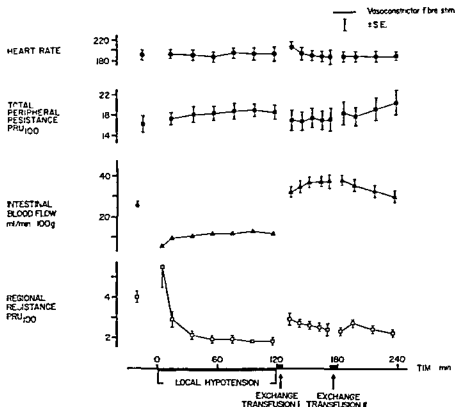


Fig 4 Heart rate total peripheral resistance intestinal blood flow and regional intestinal flow resistance during and after a mimicked shock situation in the intestine (local intestinal hypotension with stimulation of the vasoconstrictor nerves) Two exchange transfusions were made as described in detail in the text (n = 5)

performed on the skeletal muscle preparation (see below) or when intestinal hypotension and/or nervous vaoconstriction was induced for only 5 min

Five experiments were performed as described in Methods under point 2 of Experimental procedures (section C) Two exchange transfusions were made in these experiments Upon releasing the partial vascular occlusion the intestinal venous blood was collected for a 5 min period and substituted with fresh blood from another cat (exchange transfusion I) 45 min later the shed intestinal venous blood was given back to the animal and an equal blood volume was withdrawn (exchange transfusion II) No animal from this experimental series died during the post hypotensive period

Fig 4 illustrates the changes in heart rate total peripheral resistance (TPR) intestinal blood flow and resistance observed in these experiments Heart rate stayed virtually constant througout while TPR tended to increase after the second exchange transfusion (*cf* Fig 1) The intestinal vascular reactions before and during hypotension were largely similar to those in the previous group (see Fig 1) After restoring intestinal perfusion pressure a sustained hyperemia was recorded and

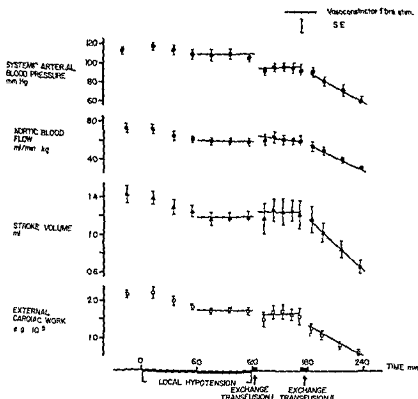


Fig 5 Arterial pressure aortic blood flow stroke volume and external cardiac work during and after local intestinal hypotension with stimulation of the vasoconstrictor nerves Two exchange transfusions were made (see text) Lines drawn by the method of least squares (n = 5)

intestinal flow resistance remained slightly above the hypotensive level throughout the rest of the experiment

Fig 5 summarizes the changes in systemic arterial pressure aortic blood flow calculated stroke volume and external cardiac work Systemic pressure fell slightly upon the first exchange transfusion but was then constant until it fell markedly after the second exchange transfusion Regression lines (method of least squares)

TABLE II k values of the regression lines for arterial blood pressure aortic blood flow stroke volume and external cardiac work illustrated in Fig 5 Mean value ± S E n = 5

	Last hypotensive hour	After exchange transfusion I	After exchange transfusion II
Arterial blood pressure	−0 055 ± 0 080	0 052 ± 0 124	−0 532 ± 0 05
Aortic blood flow	−0 027 ± 0 033	−0 085 ± 0 069	−0 436 ± 0 107
Stroke volume	−0 0003 ± 0 0009	0 0002 ± 0 0071	−0 0091 ± 0 0016
External cardiac work	−0 0007 ± 0 0015	0 0011 ± 0 0008	−0 0139 ± 0 0017

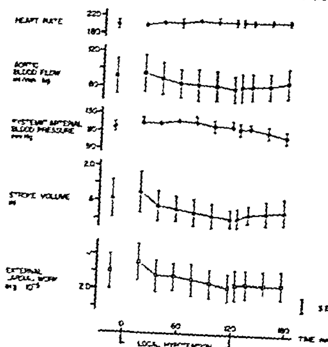


Fig 6 The effects of a period of reduced perfusion pressure in a skeletal muscle vascular bed on heart rate aortic blood flow arterial blood pressure stroke volume and external cardiac work (n = 3)

for the systemic blood pressure *vs* time were calculated 1) for the late half of intestinal hypotension 2) for the period between exchange transfusions and 3) for the period after exchange transfusion II (Fig 5) The k value of the regression lines were almost identical during the first two periods (see Table II) but became significantly changed ($p < 0.05$) after the second exchange transfusion as compared to the previous ones reflecting an accentuated pressure fall Aortic flow calculated stroke volume and external cardiac work exhibited similar response patterns (Table II) being all significantly reduced 15 min after exchange transfusion II ($p < 0.05$) also the k values of their regression lines were significantly changed ($p < 0.05$)

Immediate postmortem macroscopic examinations revealed hemorrhagic mucosal ulcerations in all the experiments of both series varying from confluent hemorrhagic areas to scattered petechial bleedings

B Skeletal muscle experiments In three experiments the arterial inflow pressure to an isolated denervated skeletal muscle vascular bed was kept at 35 mm Hg for 2 h which reduced regional blood flow from about 6 5 to 2 0—2 5 ml/100 g×min. Regional flow resistance (PRU_{100}-units) fell from 17 5 to 13 5 immediately upon the pressure drop but increased to about 15 5 during the subsequent hypotensive period. Upon pressure restoration muscle blood flow and resistance normalized rapidly Fig 6 illustrates the accumulated data on cardiac function from these animals subjected to prolonged skeletal muscle hypotension It is evident that no apparent changes occurred either during or after the hypotension Neither were any bleedings observed in the intestinal mucosa or in the skeletal muscles subjected to hypotension

Discussion

As pointed out in the introduction the present study was carried out to obtain further evidence of substances released from the gut during low flow which could explain the general cardiovascular derangement earlier observed (Haglund and Lundgren 1972 a b 1973 Haglund 1973 Åhren and Haglund 1973) Further the study aimed at exploring in which way the cardiovascular system was affected by the assumed blood borne material

To that end the experiments were so designed as to lower the perfusion pressure only to the small intestine of the cat while the regional vasoconstrictor fibres were stimulated The liver was bypassed by cannulating the intestinal vein and by returning the intestinal venous blood to the animal *via* the jugular vein directly to the right heart In fact the heart was used as a denervated *in situ* bioassay organ for cardioactive substances since its adrenergic supply was cut the adrenals denervated and the vagal nerves were blocked by atropine

The experiments in which exchange transfusions were performed (Fig 4 and 5) demonstrated clearly that a release of bloodborne material occurred in the intestine causing a pronounced and rapid reduction of most of the recorded parameters Such deteriorations of the cardiovascular system were never observed after a 2 h period of greatly reduced skeletal muscle flow (Fig 6) or when the immediate post occlusion intestinal venous effluent was substituted with fresh blood (Fig 4 and 5)

As regards the cause of the cardiovascular deterioration observed it seems clear that at least the major cause of the rapidly falling arterial pressure was of cardiac origin This conclusion is substantiated by the observed decline in aortic flow in maximal dP/dt in calculated stroke volume and external cardiac work in face of a largely constant heart rate and if anything a rising left ventricular end diastolic pressure The rapid onset of the cardiovascular derangement also favours a cardiac genesis (Fig 3) Furthermore in the second series of experiments the cardiac parameters declined in the face of a rising total peripheral resistance (Fig 4 and 5) probably induced by a reflex increase of vasoconstrictor fibre activity elicited from the baro and chemoreceptors

The failing cardiac performance could not be ascribed to a reduced venous return lowering the ventricular filling pressure since central venous pressure was if anything slightly elevated in the posthypotensive period Further it seems very unlikely that a rapid enough accumulation of blood would occur peripherally since the vasoconstrictor fibre activity appeared to increase which would counteract pooling The present data therefore indicate that the cardiovascular deterioration was caused by bloodborne substances released into the blood from the small intestine and exerting a depressing effect on cardiac performance

As mentioned above the cardiovascular derangement seen after a period of intestinal low flow started rapidly after restoring the intestinal perfusion pressure (Fig 3) probably far more rapidly than during regular hypotensive shock since the hypoxic intestinal blood bypassed the liver in the present experiments The liver probably in part protects the animal by clearing the blood from toxic substances,

On the other hand, also the liver suffers from relative ischemia during shock, probably reducing its protective capacity (Selkurt 1959).

One may then pose the question which substance(s) are responsible for the observed cardiovascular changes. In previous studies of this type of cardiovascular deterioration the arterial blood pH was followed throughout during and after periods of local intestinal hypotension but did not change significantly (Haglund and Lundgren 1972 a, b; Ahrén and Haglund 1973), probably due to the continuous bicarbonate addition given. It was also shown that osmolarity and potassium concentration of the intestinal venous plasma was within normal limits immediately after the regional hypotension (Fara and Haglund 1973). Thus neither arterial blood pH, osmolarity nor potassium concentration appear to be responsible of the recorded cardiovascular deterioration.

A release of vasoactive substances from the dog intestine after periods of total blood flow obstruction was reported by Kobold and Thal (1963) who found increased levels of bradykinin, serotonin, histamine and a polypeptid-like substance in the intestinal venous blood. Vasoactive polypeptides from the intestine has also been reported by Said and Mutt (1971) and by Lefer (1970). The polypeptide found by Lefer is reported to have a negative inotropic effect on the heart ('the myocardial depressant factor', Lefer 1970, Glenn and Lefer 1971) but is assumed to be produced in the pancreas as a result of an increased lysosomal fragility during ischemia (Glenn and Lefer 1970). Such a factor cannot explain the present findings since most of the pancreas was extirpated and the small remnant was perfused at normal arterial pressure. Bounous and McArdle (1969) have, on the other hand, called attention to the possible release of lysosomal enzymes from the ischemic small intestine.

As pointed out in the introduction, endotoxin (Fine *et al.* 1959, Fine 1967) is probably not responsible for the present observations, since the acute fatal effect of endotoxin in the cat is due to a pulmonary vasoconstriction, markedly raising right atrial pressure (Kuida *et al.* 1961, Greenway and Murthy 1971). No such events were ever recorded in the present study.

In an earlier study from this laboratory a release of serotonin from the cat small intestine was demonstrated following regional intestinal and general hemorrhagic hypotension (Fara and Haglund 1973). This is probably still another indication of a serious nutritional disturbance in mucosal-submucosal sections of the intestinal wall. Since, however, serotonin is reported to increase both heart rate and contraction force in the isolated cat heart (Erspamer 1966), it seems unlikely that serotonin contributes to the present cardiac failure. In animals with intact vagal innervation, however, serotonin depresses heart function reflexly (Erspamer 1966) and in such a situation the increased serotonin concentration in plasma may be important.

From the discussion above it is evident that several substances have been reported to be released from the ischemic intestine and claimed to be the 'intestinal factor' (Lillehei 1957). It seems, however, *a priori* most unlikely that one single substance should be responsible for the cardiovascular deterioration seen after periods of

regional intestinal hypotension or general hemorrhagic shock (Haglund and Lundgren 1972 b 1973 Haglund 1973) The small intestinal mucosa of the cat is severely damaged after 2—2 5 h of local intestinal hypotension particularly when combined with intermittent or continuous stimulation of the vasoconstrictor nerves (Haglund and Lundgren 1972 b Ahren and Haglund 1973) as well as after hemorrhagic hypovolemia (Haglund 1973) A total disintegration particularly of the villous tips was often seen at microscopic examinations a picture closely resembling that reported from man in shock (Ming 1965) and also in the shocked dog (Chiu *et al* 1970) It seems reasonable to assume that the severely damaged mucosal tissue releases into the blood stream several substances and products of tissue destruction with a negative effect on cardiac performance

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References

Appelgren K L and D H Lewis Capillary flow and capillary transport in dog skeletal muscle in hemorrhagic hock *Europ Surg Res* 1972 4 29—45

Ahren C and U Haglund Mucosal lesions in the small intestine of the cat during low flow *Acta physiol scand* 1973 88 541—550

Bounous G and A H McArdle Release of intestinal enzymes in acute mesenteric ischemia *J Surg Res* 1969 9 339—346

Carter D and A Einheber Intestinal ischemic shock in germ free animals *Surg Gynecol Obstet* 1966 122 66—76

Chiu C J A H McArdle R Brown H J Scott and F N Gurd Intestinal mucosal lesion in low flow states *Arch Surg* 1970 *101* 478—483

Erspamer V Peripheral physiological and pharmacological actions of indoleakylamines in *Handbook of Pharmac logy XIX* 5-hydroxytryptamine and related indolealk lamines pp 278—280 Springer Berlin 1966

Fara J and U Haglund Release of serotonin from the small intestine follo ing h potension in the cat. *Europ Surg R s* 1973 In press

Fine J E D Frank H A Ravin S H Rutenberg and F B Schweinburg The bacterial factor in traumat c shock *New Engl J Med* 1959 *260* 214—220

Fine J The intestinal circulation in shock *Gastroente ology* 1967 52 454—458

Folkow B and E Neil *C rculation* Oxford Un ersity P ess New York 1971

Glenn T M and A M Lefer, Role of lysosomes in the pathogenesis of splanchnic ischemia shock in cats *Ci culat Res* 1970 27 783—797

Glenn T M and A M Lefer Signif cance of splanchnic proteases in the production of a toxic factor in hemorrhagic shock *Ci culat Res* 1971 29 338—349

Greenway C V and L S Murthy Mesenteric vasoconstriction after endotoxin administration in cats p treated with aspirin *B it J Pha ma ol* 1971 43 259—269

Haglund U Va ular actions in the small intestine of the cat during hemorrhag *Acta physiol scand* 1973 89 129—141

Haglund U and O Lundgren Reactions w thin co secuti e vascular sect ons of the small intest ne of the cat during prolonged hypotension *Acta phys ol cand* 19 a 84 151—163

Haglund U and O Lundgren The effects of vasoconstrictor fib e timulat on on the con ecuti e vascular sections of the small intest ne of the cat dur ng prolonged regional h potens on *Acta physiol ca d* 1972 b 85 547—558

Haglund U and O Lundgren The effects of vasoconstr ctor fibre stimulation on consecutive sections of cat small intestine during hemorrhagic hypotension *Acta phys l sca d* 19 3 88 95—108

KOBOLD E E and A P THAL, Quantitation and identification of vasoactive substances liberated during various types of experimental and clinical intestinal ischemia *Surg Gynec Obstet* 1963 *117* 315—322

KLIDA H Discussion of "the intestinal circulation in shock *Gastroenterology* 1967 *52* 458—460

KLIDA H R. P GILBERT L. B HINSHAW J G BRUNSON and M B VISSCHER Species differences in effect of gramnegative endotoxin on circulation *Amer J Physiol* 1961 *200* 1197—1202

LEFER, A. M. Role of a myocardial depressant factor in the pathogenesis of circulatory shock *Fed Proc* 1970 *29* 1836—1847

LILLEHEI R C., The intestinal factor in irreversible hemorrhagic shock *Surgery* 1957 *42* 1043—1054

LINDEN R. J The heart ventricular function *Anaesthesia* 1968 *26* 566—584

MING S C Hemorrhagic necrosis of the gastrointestinal tract and its relation to cardiovascular status *Circulation* 1965 *32* 332—341

SAID D I and V MUTT Potent peripheral and splanchnic vasodilator peptide from normal gut *Nature* (Lond) 1970 *225* 863—864

SELKURT E. E Intestinal ischemic shock and the protective role of the liver *Amer J Physiol* 1959 *197* 281—285

SELKURT E E Status of investigative aspects of hemorrhagic shock *Fed Proc* 1970 *29* 1832—1835

SIEGEL S *Nonparametric statistics for the behavioral sciences* McGraw Hill Book Company New York 1956

Acta physiol scand 1973 89 571—579

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Parallelism between Mechanical and Metabolic Responses to Cholecystokinin and Prostaglandin E in Extrahepatic Biliary Tract

By

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Abstract

ANDERSSON K E, R ANDERSSON P HEDNER and C G A PERSSON *Parallelism between mechanical and metabolic responses to cholecystokinin and prostaglandin E_2 in extrahepatic biliary tract* Acta physiol scand 1973 *89* 571—579

The mechanical effects of the prostaglandins E_1 E_2 and F_{2} were investigated *in vitro* in isolated preparations of guinea pig gallbladder cat gallbladder and cat sphincter of Oddi The actions of the prostaglandins and particularly the effects of prostaglandin E_2 proved similar to those of cholecystokinin *i e* contraction of the gallbladder and relaxation of the sphincter of Oddi were produced the responses were not influenced by tetrodotoxin or by blockade of adrenoceptors or cholinoceptors In the anesthetized cat prostaglandin E_2 increased the intraluminal gallbladder pressure and decreased the resistance to flow through the sphincter of Oddi The contractile responses in guinea pig gallbladder after prostaglandin E_2 were preceded by a decrease in the tissue levels of cyclic AMP and an activation of phosphodiesterase The relaxation produced by prostaglandin E_2 in the cat sphincter of Oddi was associated with an increase in the cyclic AMP content of the tissue despite an increased phosphodiesterase activity These metabolic changes were similar to those observed in response to cholecystokinin The similarities in mechanical and metabolic effects between prostaglandin E_2 and cholecystokinin and the possible role of prostaglandin E_2 in mediation of the action of cholecystokinin on the extrahepatic biliary tract are discussed

Prostaglandins of the E and F types have been shown to be normally present in the gastrointestinal tract of man and various animals (for reviews see Bennett and Fleshler 1970 Bennett 1972) They are known to be potent stimulators of the motility of the small intestine it has been suggested that they play a role in the regulation of intestinal function but their physiological significance is not known (Bennett and Fleshler 1970) Several other effects are produced by the ubiquitous prostaglandins among them the ability to reproduce or decrease the effects of polypeptide hormones by interfering with the level of cyclic adenosine 3 5 monophosphate (cAMP) in the target cell (Shio *et al* 1971 Higgins and Braunwald 1972)

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KOBOLD E E and A P THAL Quantitation and identification of vasoactive substances liberated during various types of experimental and clinical intestinal ischemia *Surg Gynec Obstet* 1963 *117* 315—322

KUIDA H Discussion of the intestinal circulation in shock *Gastroenterology* 1967 *52* 458—460

KUIDA H R P GILBERT L B HINSHAW J G BRUNSON and M B VISSCHER Species differences in effect of gramnegative endotoxin on circulation *Amer J Physiol* 1961 *200* 1197—1202

LEFER A M Role of a myocardial depressant factor in the pathogenesis of circulatory shock *Fed Proc* 1970 *29* 1836—1847

LILLEHEI R C The intestinal factor in irreversible hemorrhagic shock *Surgery* 1957 *42* 1043—1054

LINDEN R J The heart ventricular function *Anaesthesia* 1968 *26* 566—584

MING S C Hemorrhagic necrosis of the gastrointestinal tract and its relation to cardiovascular status *Circulation* 1965 *32* 332—341

SAID D I and V MUTT Potent peripheral and splanchnic vasodilator peptide from normal gut *Nature* (Lond) 1970 *225* 863—864

SELKURT E E Intestinal ischemic shock and the protective role of the liver *Amer J Physiol* 1959 *197* 281—285

SELKURT E E Status of investigative aspects of hemorrhagic shock *Fed Proc* 1970 *29* 1832—1835

SIEGEL S *Nonparametric statistics for the behavioral sciences* McGraw Hill Book Company New York 1956

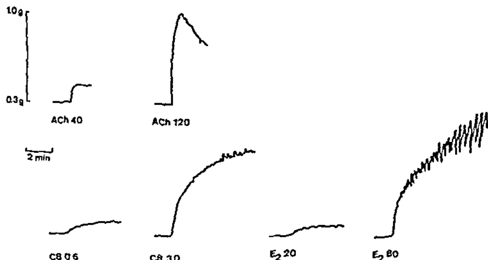


Fig 1 Responses of an isolated guinea pig gallbladder strip to threshold and submaximum concentrations of acetylcholine (Ach) C8-CCK (C8) and prostaglandin E (E₂) The drug concentrations are given in ng per ml

Results

Effects on the gallbladder in vitro

A *Mechanical effects in the guinea pig gallbladder* The prostaglandins E_1 E and $F_{2\alpha}$ all produced contraction in the isolated gallbladder strip Threshold and maximum effects were obtained in the concentration ranges 10^{-} —10^{-3} g/l for PGE_1 4×10^{-6}—4×10^{-4} g/l for PGE and 4×10^{-6}—4×10^{-3} g/l for PGF_{α}

The sensitivity to the prostaglandins varied between different preparations usually the contractile response to a given prostaglandin concentration had a tendency to increase during the course of an experiment

The amplitude of the elicited contracture was related to the prostaglandin concentration in the bath The relative potencies of the prostaglandins determined by cumulative dose response evaluations varied in the different gallbladders Potency comparisons were made between 20 and 80 per cent of maximum contractile tension PGE was always the most potent of the investigated prostaglandins On a weight basis it was 2—5 times more active than PGE_1 and 5—20 times more active than PGF_{α} On the same basis of comparison PGE was 5—10 times more active than acetylcholine but only 1/3—1/30 as active as C8 CCK

The contractile responses to the prostaglandins had certain characteristics At low concentrations the prostaglandin induced contracture developed slowly compared with the response elicited by a low concentration of acetylcholine (Fig 1) This difference was less pronounced but still quite obvious when high concentrations of the prostaglandins and acetylcholine were used C8 CCK evoked a response similar to that produced by the prostaglandins (Fig 1)

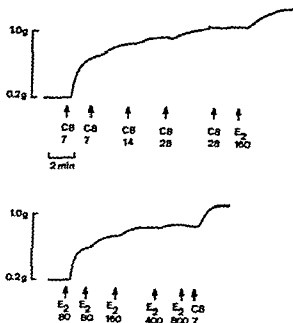


Fig 2 Combined effects of C8 CCK and prostaglandin E_2 on isolated guinea pig gallbladder Upper trace additional effect of prostaglandin E_2 (E_2) on a preparation maximally contracted by cumulative doses of C8 CCK (C8) Lower trace additional effect of C8-CCK (C8) on a preparation maximally contracted by cumulative doses of prostaglandin E_2 (E)

In about half of the number of preparations contracted by any of the prostaglandins rhythmic changes in tension were observed especially when high prostaglandin concentrations were used This was also seen after addition of C8 CCK although less pronounced but not after acetylcholine (Fig 1)

By gradually increasing the bath concentration of one of the prostaglandins investigated a maximum tension response to this agent was obtained in the gallbladder strip Addition of another prostaglandin did not increase the contractile amplitude above the level already attained However administration of C8 CCK or acetylcholine even in low concentrations produced a distinct further increase in tension (Fig 2) Similarly when a maximum contracture response to C8 CCK had been elicited addition of a prostaglandin or acetylcholine caused a further increase in tension (Fig 2) In contrast when a maximum response to acetylcholine had developed none of the prostaglandins nor C8 CCK were able to elicit an increase in contractile amplitude

Neither atropine 10^{-3} g/l nor tetrodotoxin 10^{-3} g/l influenced the responses to the prostaglandins or to C8 CCK The responses to acetylcholine were completely inhibited by this amount of atropine but were unaffected by tetrodotoxin The mechanical effects of the prostaglandins C8 CCK and acetylcholine were all unaffected by phenoxybenzamine 10^{-4} g/l and propranolol 10^{-4} g/l

B *Metabolic effects in the guinea pig gallbladder* 30 s after the administration of PGE_1 3.3×10^{-4} g/l to the bathing solution the isometric tension of the gallbladder strips had increased by (mean and S E) 5.3 ± 1.1 dyn ($p < 0.001$ $n = 20$) Then the preparations were rapidly removed from the bath and frozen by immersion in

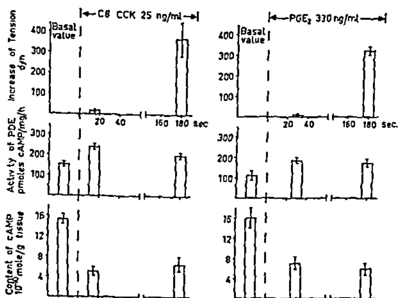


Fig 3 Means of isometric tension content of cAMP and activity of phosphodiesterase in the guinea pig gallbladder *in vitro* before and 30 and 180 s after the administration of prostaglandin E_2 4×10^{-4} g/l to the bath The bars refer to S E The corresponding responses to C8 CCK obtained in a previous investigation (Andersson *et al* 1972 a) are included for comparison

liquid freon In these preparations the activity of PDE had increased from 111 0 ± 23 7 to 184 6 ± 14 4 pmole/mg/h of cAMP hydrolyzed ($p < 0.01$ n = 8) The cAMP content had decreased from 1 6 ± 0 2 to 0 7 ± 0 2 nmole/g wet weight ($p < 0.001$ n = 12) 180 s after the addition of PGE to the bath the tension of the preparations had increased by 319 2 ± 5 4 dyn ($p < 0.001$ n = 14) The activity of PDE was then 171 1 ± 20 9 pmole/mg/h of cAMP hydrolyzed ($p < 0.05$ n = 8) and the content of cAMP 0 6 ± 0 2 nmole/g wet weight ($p < 0.001$ n = 12) In Fig 3 these effects are illustrated and compared with those produced by C8 CCK (Andersson *et al* 1972 a)

C *Mechanical effects in the cat gallbladder* Strips from cat gallbladder contracted consistently in response to PGE and PGF $_\alpha$ PGE₁ generally caused a contraction but in a few preparations this prostaglandin decreased the basal tension and relaxed contractures produced by C8 CCK The relative potencies of PGE_1 PGE PGF $_\alpha$ C8 CCK and acetylcholine were roughly similar to those obtained in the guinea pig gallbladder Also in the cat gallbladder the contractile responses to the prostaglandins were unaffected by atropine 10^{-5} g/l tetrodotoxin 10^{-3} g/l phenoxybenzamine 10^{-4} g/l and propranolol 10^{-4} g/l

Effects on the cat sphincter of Oddi in vitro

A *Mechanical effects* All the preparations of isolated sphincter of Oddi used

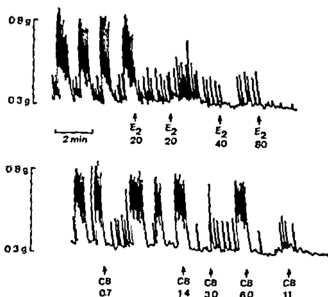


Fig 4 Effects of prostaglandin E_2 (E_2) and C8 CCK (C8) on the spontaneously active cat sphincter of Oddi *in vitro* The drug concentrations are given in ng per ml

experiments exhibited spontaneous activity PGE_1 and PGE in concentrations 0 2—20×10⁴ consistently inhibited the sphincter activity as did C8 CCK 10^{-6}—10^{-5} g/l (Fig 4) PGF$_{2\alpha}$ 1—20×10⁴ g/l had dual effects on the sphincter In about half of the preparations the activity was inhibited in the others a short lasting contraction was induced The relative potencies of the prostaglandins in producing inhibition of the spontaneous sphincter activity were not determined mainly because of the difficulties involved in the quantification of this response (Persson 1971 a) Inhibition of spontaneous activity was usually acompanied by a decrease in basal tension of the sphincter Pretreatment of the preparations with atropine 10^{-3} g/l tetrodotoxin 10^{-3} g/l and propranolol 5×10^{-4} g/l had no effect on the responses to PGE or C8 CCK Tetrodotoxin stimulated the activity of the sphincter as previously reported (Persson 1971 b)

B *Metabolic effects* The PGE induced relaxation of the sphincter of Oddi was associated with an increase in the content of cAMP in the preparation From a control value of 2 2±0 5 nmole/g wet weight the cAMP level increased by 1 35±0 5 nmole/g ($p<0\,05$ $n=6$) 30 s after the addition of PGE 4×10^{-4} g/l to the bath By then the PDE activity had increased from 40 3±2 6 to 48 2±5 3 pmole/mg/h of cAMP hydrolyzed ($p>0\,05$ $n=6$)

Effects on the cat gallbladder and sphincter of Oddi in vivo

In 3 of the 5 anesthetized cats PGE (3—5×10^{-6} g/kg b w) given iv increased the gallbladder pressure and decreased the resistance to flow through the sphincter of Oddi (Fig 5) In 2 cats however PGE increased the resistance to flow through the sphincter while contracting the gallbladder thus differing from C8 CCK which consistently had an inhibitory effect on the choledochoduodenal junction and a stimu

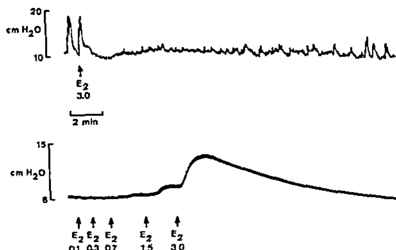


Fig 5 Resistance to flow in the perfused sphincter of Oddi (upper recording) and intraluminal gallbladder pressure (lower recording) in two anesthetized cats after the i v administration of prostaglandin E_2 (E_2) The doses are given in μg per kg b wt.

lant effect on the gallbladder In all the cats PGE produced a marked hypotension

Discussion

The present experiments showed that the mechanical effects of C8 CCK on the extrahepatic biliary tract could be mimicked by the prostaglandins E_1 E and F a and of these most consistently by PGE Similar to the actions of C8 CCK (Persson 1971 a 1973 Amer 1972 a) the prostaglandin induced responses were not influenced by pre treatment with atropine or by effective blockade of the adrenoreceptor functions in the gallbladder and in the sphincter of Oddi These findings seem to exclude involvement of cholinoceptors and adrenoceptors in the mediation of the effects Moreover, the inability of tetrodotoxin to influence the effects of the prostaglandins suggests that these substances act directly on the smooth muscle cells without involvement of neuronal elements Direct effects of prostaglandins E_1 and E have previously been demonstrated in gastrointestinal smooth muscle (Bennett Eley and Scholes 1968 a b Bennett Murray and Wyllie 1968)

Not only *in vitro* but also *in vivo* PGE and C8-CCK produced similar effects on the cat gallbladder and sphincter of Oddi contracting the gallbladder and relaxing the sphincter However in a few experiments contraction of both gallbladder and sphincter of Oddi was observed after intravenous administration of PGE It can not be excluded that the pronounced hypotensive effect of PGE influenced the response of the sphincter of Oddi in these cases as PGE always produced relaxation in the isolated sphincter preparations

The parallelism between the effects of PGE$_1$ and C8 CCK was also found when the metabolic actions were compared. PGE$_1$ decreased the cAMP level in the guinea pig gallbladder and stimulated the activity of PDE. The reduction of the cAMP content of the muscle might be explained by the prostaglandin induced activation of PDE. In the cat sphincter of Oddi, on the other hand, PGE$_1$ increased the intracellular level of cAMP. This was probably achieved by stimulation of adenyl cyclase as the activity of PDE was not reduced. The same pattern of responses was induced by C8 CCK in both organs as previously reported (Andersson *et al.* 1972 a, b), even the size of the metabolic responses proved closely similar for the two agents. These findings suggest that hormones and prostaglandins influence the level of cAMP in a target organ not only by interfering with adenyl cyclase (Shio *et al.* 1971, Hinman 1972) but also—which seems to be less commonly observed—by changing the activity of PDE. The metabolic effects evoked by PGE$_1$, similar to those induced by C8 CCK (Andersson *et al.* 1972 a, b), were found to precede the mechanical responses of both gallbladder and sphincter of Oddi, but it is not quite clear whether any causal relation exists between the two events. It has been suggested (Andersson and Nilsson 1972) that cAMP, by stimulating the calcium uptake in a microsomal fraction of smooth muscle, is one of the factors involved in the regulation of the intracellular concentration of free calcium ions necessary for the contractile activity. Thus a reduction of the cAMP content of the cell might contribute to the contraction and an increased cAMP level to the relaxation processes in the smooth muscle cells (Andersson *et al.* 1972 c, Andersson 1973). On the other hand, evidence has been presented that in rat uterus contraction induced by adrenaline after *α* receptor blockade, or by oxytocin, was not associated with changes in the levels of cAMP in the muscle (Polacek and Daniel 1971).

In the extrahepatic biliary tract PGE$_1$ was thus able to reproduce both the mechanical and metabolic effects of C8 CCK. This poses the question whether PGE$_1$ constitutes a link in the series of events that lie between the contact of C8 CCK with the target cell and its eventual effect. Such a function for prostaglandins has been proposed for part of the oxytocin induced contraction of the uterus (Vane and Williams 1972) and for the effects of several polypeptide hormones—e.g. ACTH (Flack and Ramwell 1972), TSH (Sato *et al.* 1972) and LH (Kuehl *et al.* 1970)—which have proved reproducible by prostaglandins. This question cannot be settled by the present experiments. However, if PGE$_1$ is a necessary intermediate step in the development of the CCK-effects in the biliary tract, it seems difficult to explain the finding that a low concentration of C8 CCK could further increase the tension level in a gallbladder strip that had been maximally contracted by PGE$_1$.

References

AMER, M. S. Studies with cholecystokinin *in vitro*. III. Mechanism of the effect on the isolated rabbit gallbladder strips. *J. Pharmacol. exp. Ther.* 1972 a. *183*, 527–534.

AMER, M. S. and G. R. MCKINNEY. Studies with cholecystokinin *in vitro*. IV. Effects of cholecystokinin and related peptides on phosphodiesterase. *J. Pharmacol. exp. Ther.* 1972 b. *183*, 535–548.

ANDERSSON R Role of cyclic AMP and Ca in mechanical and metabolic events in isometrically contracting vascular smooth muscle *Acta physiol scand* 1973 87 84—95
ANDERSSON R and K NILSSON Cyclic AMP and calcium in relaxation in intestinal smooth muscle *Nature (Lond)* 1972 238 119—120
ANDERSSON K E R ANDERSSON and P HEDNER Cholecystokinetic effect and concentration of cyclic AMP in gall bladder muscle *in vitro* *Acta physiol scand* 1972 a 85 511—516
ANDERSSON K E R ANDERSSON P HEDNER and C G A PERSSON Effect of cholecystokinin on the level of cyclic AMP and on mechanical activity in the isolated sphincter of Oddi *Life Sci* 1972 b 11 723—732
ANDERSSON R L LUNDHOLM E MOHME LUNDHOLM and K NILSSON Role of cyclic AMP in mechanical and metabolic events in smooth muscle *Adv Cyclic Nucleotide Res* Ed P Greengard R Paoletti and G A Robinson. Raven Press New York 1972 c 1
BENNETT A K G ELEY and G B SCHOLES Effects of prostaglandins E_1 and E_2 on human guinea pig and rat isolated small intestine *Brit J Pharmac* 1968 a 34 630—638
BENNETT A K G ELEY and G B SCHOLES Effects of prostaglandins E_1 and E_2 on intestinal motility in the guinea pig and rat *Brit J Pharmac* 1968 b 34 639—647
BENNETT A J G MURRAY and J H WYLLIE Occurrence of prostglandin E_2 in the human stomach and a study of its effects on human isolated gastric muscle *Brit J Pharmac* 1968 32 339—349
BENNETT A. and B FLESHLER Prostaglandins and the gastrointestinal tract *Gastroenterology* 1970 59 790—800
BENNETT A Effects of prostaglandins on the gastrointestinal tract In *The Prostaglandins* Ed S M M Karim MTP Co Ltd Oxford 1972 205—221
FLACK J D and P W RAMWELL A comparison of the effects of ACTH cyclic AMP dibutyryl cyclic AMP and PGE_2 on corticosteroidogenesis in vitro *Endocrinology* 1972 90 371—377
HIGGINS C B and E BRAUNWALD The prostaglandins Biochemical physiologic and clinical considerations *Amer J Med* 1972 53 92—112
HINMAN J W Prostaglandins *Ann Rev Biochem* 1972 41 161—178
KAKIUCHI S and T W RALL The influence of chemical agent on the accumulation of adenosine 3 5 phosphate in slices of rabbit cerebellum *Molecular Pharmacol* 1968 4 367—378
KUEHL F A J L HUMES J TARNOFF V J CIRILLO and E A HAM Prostaglandin receptor site Evidence for an essential role in the action of luteinizing hormone *Science* 1970 169 883—886
KUKOVETZ W R and G POCH Inhibition of cyclic 3 5 nucleotide phosphodiesterase as a possible mode of action of papaverine and similarly acting drugs *Naunyn-Schmiedeberg's Arch exp Path Pharmacol* 1970 267 189—194
PERSSON C G A Adrenoceptor functions in the cat choledochoduodenal junction in vitro *Brit J Pharmac* 1971 a 42 447—461
PERSSON C G A Excitatory effect of tetrodoxin on an isolated smooth muscle organ *J Pharm Pharmac* 1971 b 23 986—987
PERSSON C G A Dual effect on the sphincter of Oddi and gallbladder induced by stimulation of the right great planchnic nerve *Acta physiol scand* 1973 87 334—343
POLACEK I and E E DANIEL Effect of alpha and beta adrenergic stimulation of the uterine motility and adenosine 3 5 monophosphate level *Canad J Physiol Pharmacol* 1971 49 988—998
SATO S M SZABO K KOWALSKI and G BURKE Role of prostaglandin in thyrotropin action on thyroid *Endocrinology* 1972 90 343—356
SHIO H J SHAW and P RAMWELL Relation of cyclic AMP to the release and action of prostaglandin *Ann N Y Acad Sci* 1971 185 327—335
VANE J R and K I WILLIAMS Prostaglandin production contributes to the contraction of the rat isolated uterus *Brit J Pharmac* 1972 45 146 P

Acta physiol scand 1973 89 580—593
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Countercurrent Multiplication of Sodium in Intestinal Villi during Absorption of Sodium Chloride

By

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Abstract

HALJAMÄE H M JODAL and O LUNDGREN *Countercurrent multiplication of Na in the intestinal villi during absorption of sodium chloride* Acta physiol scand 1973 *89* 580—593

The sodium and the potassium concentrations in the villous tissue expressed as μg Na or K per mg tissue protein were determined *in vivo* along the villous length during the absorption of isotonic saline solutions The sodium concentrations was found to be 3—4 times higher at the tip than at the villous base The addition of 30 mmol glucose to the incubation medium resulted in a significant increase of the sodium concentration along villous length while this gradient was drastically decreased by ischemia or intense vasodilatation as well as by intra arterial infusion of ouabain The intracellular sodium and potassium concentrations at the villous base were calculated to 21 and 125 mEq/l respectively during isotonic saline absorption The extracellular sodium concentration at the villous tip was estimated to 300—900 mEq/l corresponding to an interstitial osmolarity of 600—1600 mOsm/l It is proposed that a counter current multiplication of sodium occurs in the villi resulting in a high interstitial osmolarity at the villous tip The functional importance of the countercurrent multiplication of sodium for the absorption of water is discussed

The transfer of solutes and water across epithelial membranes is closely linked and it is believed that a difference in osmolarity across the membrane is a prerequisite for the movement of water As regards intestinal absorption this relationship between solute and water has been convincingly demonstrated *in vitro* as well as *in vivo* (for review see Schultz and Curran 1968)

Several models have been proposed to explain the experimental observations that water transport occurs in the absence or against an osmotic pressure difference The currently most accepted hypothesis seems to be that proposed by Curran and MacIntosh (1962) often referred to as the double membrane hypothesis and based on a theoretical framework of non equilibrium thermodynamics This hypothesis involves 2 membranes in series in a 3 compartment system An active solute transport occurs across the first membrane with a low solute permeability creating an osmotic force that induces a transfer of water This in turn leads to an increased hydro

static pressure in the second compartment placed between the two membranes which then drives solute and solvent across the second more permeable membrane

Concerning the anatomical counterparts of this model it has been proposed that the second compartment is actually made up by the space between the epithelial cells and the basement membrane, the third one being constituted by the interstitial space of the *lamina propria* The intercellular localisation of the second compartment with a high osmolarity has been particularly stressed by Diamond (1968) in his local osmotic theory , which may be regarded as a special case of the double membrane hypothesis However any experimental results which demonstrate the presence of a hypertonic intercellular space in the small intestine have not been reported

As regards the small intestine the situation is further complicated by the fact that the vascular compartment which constitutes the major transport vehicle for most solutes seems to allow for a countercurrent exchange diffusion of easily diffusible solutes in the hairpin vascular loops characterizing the villous blood supply (see *e g* Lundgren 1967) It may then be possible during *e g* NaCl absorption that a slight difference of solute concentration between the two limbs of the villous vascular loop may be multiplied along the villous length If so this countercurrent multiplica tion would in a similar way as in the kidney papillae create an increasingly hyper osmotic interstitial fluid towards the villous tips

To test this hypothesis the distribution of sodium along the villous length was in vestigated during absorption of sodium chloride during normal enhanced and greatly reduced blood supply to the villous vascular loops hemodynamic changes which would be expected to affect the efficiency of the proposed countercurrent exchanger Furthermore the active epithelial transfer capacity for sodium was in creased by adding glucose to the sodium chloride solution or decreased by giving ouabain i.a A preliminary report of this study has been published (Haljamae *et al* 1971)

Methods

A Operative and experimental procedures The experiments were performed on cats anes thetized i.v with chloralose (50 mg/kg b wt) after induction with ether The animals had been deprived of food for 24 h and had no obvious signs of intestinal infection The operative proce dures and the recording of blood flow were largely similar to that described earlier (Haglund Jodal and Lundgren 1973) Briefly total venous outflow from 3—5 sympathetically isolated and denervated jejunal segments was measured by a drop recorder ordinate writer unit Mean arterial pressure was measured from the left femoral artery by a pressure transducer Each jejunal segment was cut open by thermocautery along the antimesenteric border and firmly mounted flat on a metal frame On top of the metal frame a plastic frame was placed forming a 20 mm × 40 mm bath that made it possible to put any desired solution (see below) in direct contact with the exposed mucosa. The temperature of the solution in the bath was controlled by a thermocouple thermometer (Electrolab Copenhagen) and kept at 37 —38 C by table lamps

The exposed mucosal surface was continuously flushed with a solution containing sodium chloride for 15 min The flushing was performed by means of an infusion pump at a rate of 1—2 ml/min Immediately after stopping the infusion the segment was briskly rinsed with approximately 40 ml of a bodywarm isotonic sucrose solution to remove the saline from the mucosa The intestinal segment mounted on its metal frame was then swiftly extirpated and momentarily frozen in liquid nitrogen The sucrose flushing and freezing of a segment required a time of 4—5 s

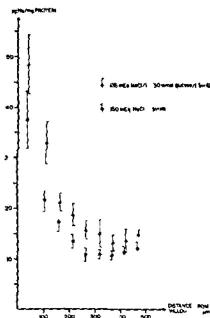


Fig 1a

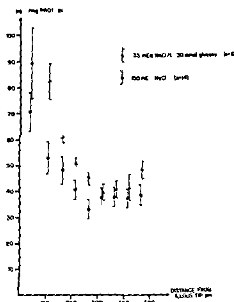


Fig 1b

Fig 1 The concentration of sodium (left panel) and potassium (right panel) in the villous tissue expres ed as µg/mg protein along the villous l ngth The intestinal mucosa was for 15 min during resting' blood flow exposed to either isotonic saline (filled circles) or to an isotonic saline solution containing 30 mmol glucose (filled triangles) Bars denote ± S E

B Tissue sectioning A flat part from the frozen intestinal ti ue v as cut out in a cryostate (System Dittes Duspiva) the surface area ranging between 50—200 mm² The tissue segment v as then mounted on a trimmed piece of ice on the microtome chuck with a few drops of water along the edges of the segment The margin of the mucosa 1 mm in width was removed since artifacts might have been caused by the v ater used for mounting of the tissue The mucosa was then sliced in 10 µm thick sections perpendicular to the long axis of the vi li The part of the mounted mucosa which had not been cut within 130 µm from the first se tion was dissected away The amount of Na and K per unit v eight protein of the intestinal tissue was then determined in 40—60 µm thick tissue slices (see below) Nine sections from different levels of the villi each 10 µm thick were placed on glass slides and stained v ith hematoxylin eosin in order to make h stological control of the sectioned tissue

C Determinations of potassium sodium and protein 4—6 slices each 10 µm thick ere put in a plastic tube together v ith 400 µl destilled water After at least 2 h equilibration at 30—35 C the tube was centrifugated and 100 µl was pipetted from the supernatant The potassium and sodium concentration of this solution was determined by means of the technique described by Haljamäe and Larsson (1968)

The ti sue in the remaining 300 µl was precipitated ith 0 5 ml 10 % perchloric acid dissolved in 1 5 ml 0 3 N NaOH for 30 min and then again precipitated in 0 5 ml 6 N HCl The purified protein was di solved in 0 5—2 0 ml 1 N NaOH depending upon the expected amount of protein and v as heated in a boiling water bath for 5 min Total protein content was then determined according to Lowry *et al* (1951) with bovine serum albumin as a standard (Sigma Stock 905—10)

D Experimental series The following fi e experimental seri s were performed at resting intestinal blood flow

1 Absorption of an isotonic solution containing NaCl and glucose (135 mEq NaCl/l 30 mmol glucose/l) c molarity around 290 mOsm/l)
2 Absorption of an isotonic saline solution (150 mEq NaCl/l)
3 Absorption of a hypertonic saline solution (225 mEq NaCl/l)
4 Ab orption of a hypotonic s line solution (75 mEq NaCl/l)

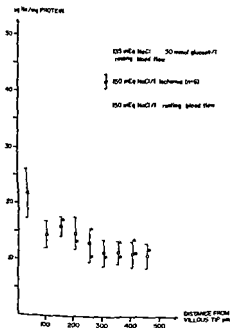


Fig 2a

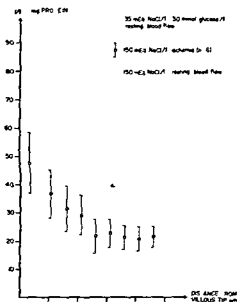


Fig 2b

Fig 2 The concentration of sodium (left panel) and potassium (right panel) in the villous tissue expressed at µg/mg protein along the villous length The intestinal mucosa was for 15 min exposed to oxygenated isotonic saline during total intestinal ischemia (open squares) As a comparison is shown the concentration of sodium and potassium, respectively along the villous length during absorption of isotonic saline (filled circles) and an isotonic saline solution containing 30 mmol glucose (filled triangles) Bars denote ± S E

5 Absorption of an isotonic saline solution during an intraarterial infusion of saline containing 10^{-4} mol ouabain/l at a rate of 0 1—0 2 ml/min, resulting in an ouabain plasma concentration of 1—2 × 10^{-5} mol/l The venous effluent f om the "absorbing" jejunal segment was in these experiments coll cted via a cannula in the draining vein to hinder systemic effects of the ouabain

Absorption experiments with an isotonic saline solution was also performed during intense intestinal vasodilatatio induced by i a infus on of isopropylnoradrenaline (20 µg/ml dissolved in saline at a rat of 5—10 g/min via a cannula in a small branch of the superior mesenteric artery

Finally one er es of experiments during absorption of isotoni saline was performed during total intestinal ischemia accomplished by complete obstruction of the ascular suppl to the segment. In th s type of experiment the al ne solution was equil brated th pure oxygen

Results

The left panel of Fig 1 illustrates the sodium content along the villous length expressed as µg Na/mg tissue protein Since tissue protein represents an indirect measure of tissue volume the sodium or potassium content expressed as in Fig 1 will below the named concentration Two types of experiments performed at resting blood flow (mean flow 29 ml/min × 100 g range 21—39) are illust in Fig 1 In one eries the intestinal mucosa was exposed to a solution c

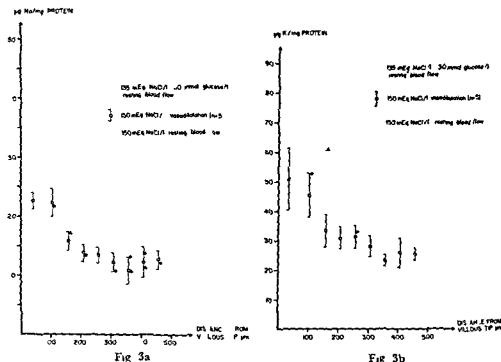
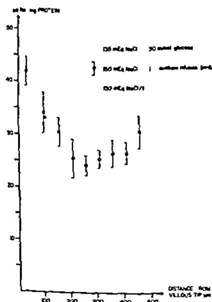


Fig 3 The concentration of sodium (left panel) and potassium (right panel) in the villous tissue expressed as $\mu g/mg$ protein along the villous length Open squares demonstrates the concentration gradient obtained when the intestinal mucosa was exposed to isotonic saline during maximal vasodilatation As a comparison is shown the concentration of sodium and potassium respectively along the villous length during the absorption of isotonic saline with glucose (filled triangles) and without glucose (filled circles) Bars denote ± S E

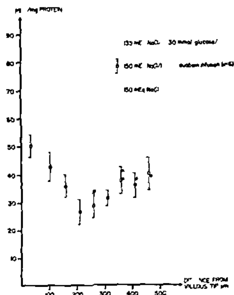
150 mEq NaCl/l H_2O for 15 min in another to a solution containing 135 mEq NaCl/l and 30 mmol glucose/l also for 15 min

During incubation with isotonic saline the sodium concentration becomes 3—4 times higher at the villous tip than at the base Adding glucose to the incubation medium resulted in an increase of the sodium concentration along the whole villous length the values on the decreasing part of the curves being significantly different ($p < 0.05$) except for the tip value —The right panel of Fig 1 illustrates the potassium concentration in the villous tissue during the same experimental conditions The concentration ratio between tip and base of the villus during absorption of isotonic saline is here slightly less than 2

The results illustrated in Fig 1 suggest that the tissue concentration of sodium during the absorption of isotonic saline is much higher at the villous tip than in the rest of the mucosa To study the importance of blood flow for the development of this uneven distribution of sodium in the villi experiments were performed both during ischemia produced by clamping the vessels of the intestinal segment and at intense vasodilatation induced by i a infusions of isopropylnoradrenaline (mean blood flow 205 ml/min × 100 g range 115—335) Absorption during interrupted



F g 4a



F g 4b

Fig 4 The concentration of sodium (left panel) and potassium (right panel) in the villous tissue expressed as μg/mg protein along the villous length Open squares illustrate the concentration patterns in the villous tissue in experiments in which isotonic saline was absorbed during intra arterial infusion of ouabain As a comparison is shown the concentration of sodium and potassium respectively along the villous length during the absorption of isotonic saline with glucose (filled triangles) and without glucose (filled circles) Bars denote ± S E

blood flow a simulated *in vitro* situation where the oxygenated isotonic saline solution would provide the absorbing epithelial cells with oxygen during the 15 min absorption period (Fig 2) resulted in a significant decrease of sodium concentration at the villous tip compared to the situation during resting blood flow ($p < 0.05$ pairing design test) The villous potassium concentration on the other hand decreased along the whole villous length the concentration ratio between tip and base being almost the same as at resting' blood flow (Fig 2 right panel)

When the blood flow during absorption of isotonic saline was maximally increased by means of i a isopropylnoradrenaline the same concentration profile in the villi as during ischemia was obtained for both sodium and potassium (Fig 3) The tip value for both sodium and potassium were significantly different from those observed at rest ($p < 0.01$ pairing design test)

In order to decrease the rate of sodium absorption ouabain a glucoside known to specifically inhibit the active transfer of sodium was infused intraarterially to an intestinal segment when exposed to an isotonic saline solution for 15 min (

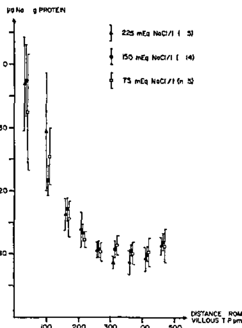


Fig 5a

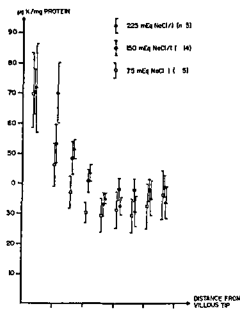


Fig 5b

Fig 5 The concentration of sodium (left panel) and potassium (right panel) in the villous tissue expressed as µg/mg protein along the villous length The intestinal mucosa was during resting blood flow exposed to three different saline solutions 225 mEq NaCl/l (filled angles) 150 mEq NaCl/l (filled circles) and 75 mEq NaCl/l (open squares) Bars denote

blood flow 26 ml/min × 100 g range 21—32) As is shown in Fig 4 the concentration of sodium markedly increased particularly at the villous base as compared to rest (*cf* Fig 1) to such an extent that the concentration gradient of sodium along the villous length was in this situation reduced the tip to base ratio being well below 2 As regards potassium the tip concentration was reduced with a largely unchanged base concentration the tip to base ratio being close to 1

The observations described so far have been obtained during absorption of *isotonic* solutions but it is wellknown that sodium absorption can take place despite relatively large variations in intraluminal osmolarity A series of experiments was therefore performed to study the villous sodium concentration during absorption of hypotonic (75 mEq NaCl/l) and hypertonic (225 mEq NaCl/l) saline (mean blood flow 34 ml/min × 100 g, range 29—42) The results illustrated in Fig 5 indicate that the sodium concentration gradient along the villous length which is created during absorption of isotonic saline may not be appreciably changed by these large shifts in luminal tonicity

Discussion

The aim of the present study was to investigate the sodium concentration measured as μg Na/mg protein along the intestinal villi during the absorption of different saline solutions at various states of villous blood flow. Since the concomitant changes in villous potassium concentration are probably secondary to the changes in extra and/or intracellular sodium content the discussion will be focused on the sodium shifts along the villi.

The sodium concentration gradient along the villous length illustrated in Fig. 1 might be caused by an uneven distribution of the epithelial capacity for active sodium transport along the villous length. However, the markedly reduced concentration gradient during interrupted blood flow strongly suggests that such a factor could account for only a minor part of the observed gradient, since the villous sodium concentration should otherwise have increased, not decreased (*cf.* Fig. 2), in the absence of a bloodborne sodium elimination. The same findings speak against the possibility that the gradient should be a consequence of a blood flow limited sodium absorption.

An alternative explanation for the high sodium concentration in the villous tip may, as outlined in the introduction, be the establishment of a countercurrent multiplication in the intestinal villi. The anatomical basis for such a mechanism is constituted by the hairpin vascular loop formed by the unbranched central villous arterial vessel closely surrounded at only 15—20 μm distance by the dense subepithelial capillary network. It has been shown that oxygen, antipyrine and probably also urea are extravascularly shunted from the central artery into the capillary network at the villous base when they reach the villi by means of the arterial blood stream (Kampp, Lundgren and Sjostrand 1968, Kampp, Lundgren and Nilsson 1968). Furthermore, krypton and fatty acids, when entering the capillary network from the intestinal lumen, diffuse in the opposite direction from the capillaries to the central artery, leading to a "hampering" of their net blood absorption (Biber, Lundgren and Svanvik 1973, Haglund, Jodal and Lundgren 1973, Jodal and Lundgren 1973 a, b, Svanvik 1973). Thus the hairpin arrangement of the intestinal villi allows a countercurrent exchange to take place both for lipid soluble substances and to a less extent also for water soluble pore bound substances. However, water itself, known to diffuse to some extent also across the endothelial cell membranes (Yudilevich and Alvarez 1967), may pass more readily then the pore bound solutes.

The high sodium content at the villous tips may, according to the countercurrent hypothesis, be explained by two possible mechanisms. The first possibility implies that the actively absorbed sodium is shortcircuited from the subepithelial capillary network to the central artery, resulting in a recirculation and concentration of sodium towards the villous tips. This would call for the presence of fenestrations or interendothelial pores in the wall also of the central arterial vessel. The increase of sodium concentration in the villous capillaries, ultimately due to the active epithelial uptake of sodium (below named "single effect", *cf.* Dicker 1970), would then be multiplied along the villous length resulting in a sodium accumulation in the villous

*

tip as in the renal papillae The second possibility implies that the increase of sodium concentration and osmolarity in the villous capillaries causes a transfer of water from the arterial vessel to the capillary network, eventually resulting in a similar sodium multiplication as described above Results obtained in a autoradiographic study of intestinal sodium absorption seems to favour the view that both mechanisms contribute (Jodal, to be published) Since water moves more quickly across cell membranes than sodium one would *a priori* expect the second mechanism to be most important

The efficiency of a countercurrent exchanger is depending on the permeability characteristics of the membranes involved in the system but also on the time available for diffusion Thus circulatory arrest or a sufficiently marked increase in linear rate of flow would both abolish or markedly decrease the villous concentration gradient The results shown in Fig 2 and 3, where such changes in rate of blood flow were induced are therefore in full agreement with the countercurrent hypothesis

In a countercurrent multiplication system the created gradient also depends on the single effect i e in this case on the capacity of the active transport mechanism for sodium in the villous epithelium Stimulation or inhibition of this mechanism would therefore be expected to affect the sodium concentration gradient along the villous length Glucose known to increase the active sodium transport both *in vitro* and *in vivo* (Schultz and Curran 1970) also resulted in a significant increase of the sodium concentration at the villous tip (Fig 1) On the other hand i a infusion ouabain in doses known to inhibit at least partly the active sodium transport hultz and Zalusky 1964) markedly reduced the concentration gradient from tip villous base (Fig 4 The increase of sodium content now observed also at the villous base was probably caused by a sodium leakage into the villous cells caused by the ouabain (Schultz Fuisz and Curran 1966)

Thus the present results strongly suggest that the creation of a marked sodium concentration gradient along the villous length depends on the existence of a blood flow Furthermore the gradient is affected by procedures that incrase or decrease the efficiency of the countercurrent multiplier while it seems to be largely unaffected by changes in the luminal sodium concentration (Fig 5 see below) It should be pointed out in this connection that the blood flow increase observed in the intestine during digestion seldom exceeds 100 per cent of control (for ref see Lundgren 1967) while intestinal blood flow was increased 7—8 times in the present study by means of i.a isopropylnoradrenaline Fig 2) It is therefore proposed that *in vivo* a countercurrent multiplication of sodium takes place in the villi during the absorption of sodium chloride with secondary important effects on water absorption as outlined below

The villous potassium concentrations recorded in the present study showed gradients of a similar type as for sodium although the tip to base concentration ratio never was as large as for sodium As pointed out above it seems probable that the observed changes in potassium concentration were secondary to alterations in sodium

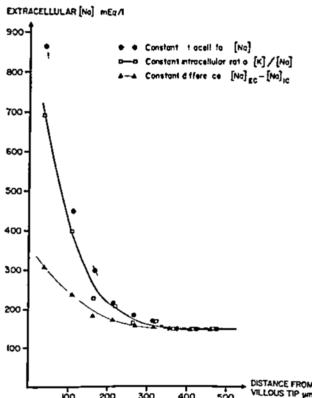


Fig 6 Theoretical calculations of the extracellular sodium concentration in the villous tissue expressed in mEq/l along the villous length The estimations are based on three different assumptions The intracellular sodium concentration [Na] the intracellular concentration ratio of potassium and sodium [K]/[Na] or the difference between extra and intracellular sodium concentration $[Na]_{EC}$-$[Na]_{IC}$ are assumed to be constant along the villous length The calculations are based on the results obtained during absorption of an isotonic saline solution containing 30 mmol glucose (Fig 1) assuming a villous interstitial space of 22 per cent

tissue content However without basic knowledge of such important variables in the villi as cell membrane permeability to potassium or *in vivo* membrane potentials it is at present impossible to explain the observed potassium shifts

The analytic methods used in the present study give the concentrations for sodium and potassium in the entire villous tissue giving no information about the distribution between intra or extracellular compartments However assuming that the extracellular concentration of sodium at the base of the villi is equal to the plasma concentration i e 150 mEq Na/l in the cat (Kjellmer 1965) that the villous extracellular space is 22 % of total tissue water (Esposito Faelli and Capraro 1972) and that the protein content in the villous tissue is 10 4 g/100 g tissue (Jodal and Lundgren unpublished observations) approximate values for the intracellular concentration of sodium at the villous base may be calculated One then obtains a value of 21 mEq/l intracellular water during the absorption of isotonic saline and a value of 40 mEq/l when also glucose is absorbed Using a value for potassium concentration in cat plasma of 4 0 mEq/l (Kjellmer 1965) and the same assumptions as above the intracellular potassium concentration would be about 125 mEq/l without and 135 mEq/l with glucose present in the incubation medium In studies *in vitro* the intracellular sodium and potassium concentrations have been estimated to about 50 mEq/l and 110 mEq/l respectively (Schultz and Zalusky 1964)

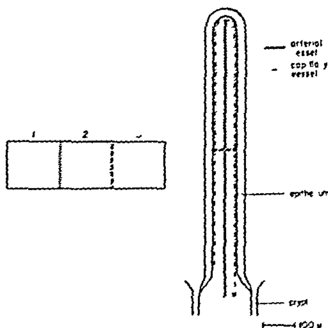


Fig 7 *Left part* The theoretical "double membrane" model for water transport as proposed by Curran and MacIntosh (1962) The shaded area denotes the second hyperosmotic compartment into which the solute is actively transported *Right part* The "double membrane" model adopted to the proposed *in vivo* situation of a countercurrent multiplication of sodium in the hair-pin vascular loops of the villus The shaded area in the lamina propria roughly corresponding to the villous tissue region with an increased sodium concentration in Fig 1 represents the anatomical counterpart to the hyperosmotic compartment in the "double membrane" model The membrane between compartments 2 and 3 in the "double membrane" model is *in vivo* constituted by the countercurrent exchanger and/or the central lymphatic lacteal

It is however much more difficult to estimate the distribution of sodium and potassium between the extra and intracellular spaces at the villous tip where the concentrations are much higher Fig 6 illustrates calculations of the villous extracellular concentration of sodium based on 3 different assumptions The theoretical curves were constructed from values obtained during absorption of a saline solution containing 30 mmol glucose per l (Fig 1) First if it is assumed that the intracellular sodium concentration is constant along the villous length one arrives at an extracellular sodium concentration as indicated by the filled circles i e the extracellular sodium concentration at the villous tip is 665 mEq/l Second assuming instead that the ratio between intracellular potassium and sodium concentrations is constant along the entire villus the calculated extracellular sodium concentration is about 690 mEq/l at the villous tip (open squares) Third one may assume a constant difference between extra and intracellular sodium concentrations along the villous length. In Fig 6 this difference is set to 110 mEq/l i e the difference present at the villous base In this case the calculated extracellular sodium concentration at the villous tip is 310 mEq/l (filled triangles

These 3 alternatives of extracellular sodium concentration in the villous tips would correspond to an interstitial osmolarity of about 1600 1275 and 575 mOsm/l respectively The first two values are surprisingly high compared with the maximal osmolarity found in the tip of the kidney papillae of man being here around 1200 mOsm/l

and probably involve an overestimation The third value correspond to an intra cellular sodium concentration of about 200 Na mEq Na/l a value which is un doubtedly too high and hence the interstitial osmolarity of 575 mO m/l i considered to be an underestimation Part of the abovementioned overstimation may reside in the assumption of a constant extracellular space along the villous length but this space must vary with *e g* the extent of filling of the central lacteal (Kalima 1971) Such factors may also explain why no significant difference was seen between the sodium concentrations at the villous tip during absorption of saline of different tonicities (Fig 5) However an uneven distribution of the villous interstitial space *cannot* explain the sodium gradient that is present also during resting conditions (Fig 1) since it would then call for an interstitial space making out 70 per cent of the total tissue volume at the villous tips This value is decidedly too high since the intestinal epithelium alone constitutes here at least 50—60 per cent of the villous volume (Jodal unpublihed observations)

As stated in the introduction the intestinal absorption of water is closely linked to the active absorption of solutes The observation that water can be absorbed in the absence of or against an osmotic pressure difference implies the existence of a tissue *compartment into which solutes are actively pumped over a membrane with low* solute permeability, resulting in a local tissue hyperosmolarity (Curran and MacIntosh 1962) as schematically illustrated in the left part of Fig 7 Since it has been demonstrated *in vitro* as well as *in vivo* that water can be ab orbed from a solution of about 400 mOsm/l (Visscher *et al* 1944 Vaughan 1960 Annegers and Wakefield 1962 Grim 1962 Schultz and Curran 1968) the hypothetical hyper osmolar compartment must contain a solution with at least that osmolarity During *in vitro* conditions in the absence of any blood flow such a high osmolarity may very well be created in the intercellular space of the villous epithelium as proposed by Schultz and Curran (1968) However it seems doubtful that such a hyperosmolarity can be maintained *in vivo* in very close approximation of the considerable blood stream in the subepithelial fenestrated capillaries which have a blood flow of the order of 40—50 ml/min × 100 g villous tissue even during resting conditions The maintenance of such a high local hyperosmolarity would call for a very large transport capacity of sodium at the tissue membrane of the epithelial cell

According to the countercurrent hypothesis the hyperosmolar compartment will have its anatomical counterpart in the villous core in which a hyperosmolarity is created by the multiplication *of a comparatively small singel effect (see shaded* area of the villus in Fig 7) The counterpart to the second membrane in the double membrane model would then be the vascular countercurrent exchanger and/or the central lymphatic lacteal in the villus having a high permeability to both water and solutes In the schematic illustration of Fig 7 this membrane has been placed in the middle of the villus roughly corresponding to the end of the decreasing part of the sodium concentration curve (*cf* Fig 1) The localization of this border will among other things be determined by the linear blood flow rate in the villous vascular bed

The hyperosmolarity in the interstitial space of the villous tip would probably lead to secondarily increased hydrostatic pressure in this region creating a pressure head for the flow of lymph along the lacteal. This fluid movement would be further facilitated by the valvelike structures situated in the wall of the central lacteal allowing only passage of fluid into the lacteal (Kalima 1971) The observation made by several workers that lymph flow increases during water absorption is in accordance with the mechanism proposed above (Simmonds 1954 Kim and Bollman 1954 Barrowman and Roberts 1967, Lee and Duncan 1968)

It could be argued that a villus may be too small a structure to contain any larger concentration differences of sodium along its length. It can however be calculated that a 94 per cent diffusion equilibrium for sodium over a 200 μm wide sheet of water is reached first after 32 s (Davson 1970 p 397) This figure should be compared to a mean transit time of plasma in the villi of only 4—6 s at the "resting level of intestinal blood flow (about 25—30 ml/min × 100 g intestine Biber Lundgren and Svanvik 1973) Thus a longitudinal diffusion of sodium in the villi would probably have only a small effect on the efficiency of the countercurrent multiplier since this would operate far more rapidly

It is hence proposed that the countercurrent multiplication of sodium, occurring during the absorption of sodium chloride is of major importance also for the absorption of water particularly when this occurs against a luminal to tissue osmotic gradient. It would be expected that the absorption of any solute that increases plasma osmolarity in the villous capillary network (e g glucose amino acids) would cause a nsfer of water from villous central artery to villous capillaries. This in turn would to a high concentration of sodium chloride in the extracellular space of the ous tip as described above enhancing water absorption.

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References

ANNEGERS J H and H WAKEFIELD Electrolyte urea and water movements across canine intestinal mucosa. *Amer J Physiol* 1962 *203* 553—556
BARROWMAN J and K B ROBERTS The role of the lymphatic system in the absorption of water from the intestine of the rat. *Quart J exp Physiol* 1967 52 19—30
BIBER, B., O LUNDGREN and J SVANVIK Intramural blood flow and blood volume in the small intestine of the cat as analyzed by an indicator-dilution technique *Acta physiol scand* 1973 *87* 391—403
CURRAN P F and J R MACINTOSH A model system for biological water transport. *Nature (Lond)* 1962 *193* 347—348
DAVSON H., *A textbook of general physiology* J & A Churchill London 1970 p 397
DIAMOND J D Transport mechanisms in the gallbladder In *Handbook of Physiology* 1968 Section 6 Vol. IV 2451—2?4
DICKER, S E. *Mechanisms of urine concentration and dilution in mammals* Edward Arnold Publ. Ltd. London 1970
ESPOSITO G., A FAELLI and V CAPRARO The extracellular space of rat intestine "in vivo" *Pflügers Arch. ges Physiol.* 1? 2 *331* ?0—?6
GIRM, E., Water and electrolyte flux rates in the duodenum jejunum, ileum and colon, and effects of osmolarity *Amer J dig D* 1962 7 1?—27

HAGLUND U M JODAL and O LUNDGREN An autoradiographic study of the intestinal absorption of palmitic and oleic acid *Acta physiol scand* 1973 89 457—481
HALJAMAE H and S LARSSON An ultra micro flame photometer for K and Na analysis of single cells and nanoliter quantities of biological fluids *Chem Instr* 1968 1 131—141
HALJAMAE H M JODAL O LUNDGREN and J SVANVIK The distribution of Na in the intestinal villi during absorption of sodium chloride *Acta physiol scand* 1971 83 283—285
JODAL M and O LUNDGREN The distribution of absorbed ^{3}H palmitic acid in the intestinal villi of the cat during various circulatory conditions *Acta physiol scand* 1973 89 318—326
JODAL M and O LUNDGREN Studies on the *in vivo* absorption of butyric acid in the small intestine of the cat. *Acta physiol scand* 1973 89 327—333
KALIMA T V The structure and function of intestinal lymphatics and the influence of impaired lymph flow on the ileum of rats *Scand J gastroenterology* 1971 6 Suppl 10
KAMPP M O LUNDGREN and N J NILSSON Extravascular shunting of oxygen in the small intestine of the cat *Acta physiol scand* 1968 72 396—403
KAMPP M O LUNDGREN and J SJOSTRAND The distribution of intravascularly administered lipid soluble and lipid insoluble substances in the mucosa and the submucosa of the small intestine of the cat. *Acta physiol scand* 1968 72 469—480
KIM K S and J L BOLLMAN Effect of electrolytes on the formation of intestinal lymph in rats *Amer J Physiol* 1954 179 273—277
KJELLMER I The potassium ion as a vasodilator during muscular exercise *Acta physiol scand* 1965 63 460—468
LEE J S and K M DUNCAN Lymphatic and venous transport of water from rat jejunum a vascular perfusion study *Gastroenterology* 1968 54 559—567
LOWRY O H N J ROSEBROUGH A LEWIS FARR and R J RANDALL Protein measurement with the Folin phenol reagent *J biol Chem* 1951 193 265—275
LUNDGREN O Studies on blood flow distribution and countercurrent exchange in the small intestine *Acta physiol scand* 1967 Suppl 303
SCHULTZ S G and P F CURRAN Intestinal absorption of sodium chloride and water In *Handbook of Physiology* 1968 Section 6 Vol III 1245—1275
SCHULTZ S G and P F CURRAN Coupled transport of sodium and organic solutes *Physiol Rev* 1970 50 637—718
SCHULTZ S G R E FUISZ and P F CURRAN Amino acid and sugar transport in rabbit ileum *J gen Physiol* 1966 49 849—866
SCHULTZ S G and R ZALUSKY Ion transport in isolated rabbit ileum *J gen Physiol* 1964 47 567—584
SIMMONDS W J The effect of fluid electrolyte and food intake on thoracic duct lymph flow in unanaesthetized rats *Aust J exp Biol* 1954 32 285—300
SVANVIK J The effect of reduced perfusion pressure and regional sympathetic vasoconstrictor activation on the rate of absorption of ^{86}Rb from the small intestine of the cat *Acta physiol scand* 1973 89 239—248
VAUGHAN B E Intestinal electrolyte absorption by parallel determination of unidirectional sodium and water transfers *Amer J Physiol* 1960 198 1235—1244
VISSCHER, M B E STANTON FETCHER JR C W CARR H P GREGOR M S BUSHEY and D E BARKER Isotop tracer studies on the movement of water and ions between intestinal lumen and blood *Amer J Physiol* 1944 142 550—575
YUDILEVICH O and O A ALVAREZ Water sodium and thiourea transcapillary diffusion in the dog heart *Amer J Physiol* 1967 213 308—314

Acta physiol. scand. 1973. 89. 594—599

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A Study of Plasma and CSF Potassium Relationship during Postnatal Development in the Cat

By

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Abstract

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The concentration of potassium in plasma and cerebrospinal fluid obtained from the cisterna magna was determined in anesthetized kittens and adult cats before and after elevation of the potassium concentration in blood by means of 1) i.v. isotonic KCl infusion for 2 h and 2) feeding with isotonic KCl for 12 h. The results suggest that in anesthetized animals a slight decrease in CSF potassium concentration occurs postnatally. Elevation of the potassium concentration in blood caused a rise in CSF potassium concentration which was very slight in all age groups but was somewhat larger in the kittens than in the adults.

In adult mammals there exists a significant difference between the potassium concentration in plasma and in cerebrospinal fluid (CSF). Furthermore, large changes in the concentration of plasma potassium, acute or chronical, induce only small change or none at all in the potassium concentration of the CSF (Bekaert *et al.* 1951, Cooper *et al.* 1955, Ames *et al.* 1965, Bradbury and Davson 1965). These findings indicate the presence of homeostatic mechanisms which tend to keep the potassium concentration of the CSF constant. The nature of these mechanisms remains obscure. Studies on the relationship between CSF and the extracellular fluid (ECF) of the central nervous system suggest that even high molecular substances pass the pia mater and the ependyma without significant resistance (Brightman 1965, 1968) and also that within the ECF ions such as potassium, calcium and sodium seem to be distributed in a manner corresponding to that of free diffusion (Brightman 1965, Pappas and Purpura 1966, Nicholls and Kuffler 1964). Thus one may assume that in adult mammals the homeostatic mechanisms tend to stabilize the ionic environment of neurons and glial cells within narrow limits.

Ontogenetic studies on the homeostatic mechanisms of electrolytes in CSF are few and no postnatal study has so far been performed on the potassium concentration gradients between plasma and CSF in the cat, nor have the effects of large changes in plasma potassium concentrations on the CSF potassium level been investigated in newborn mammals.

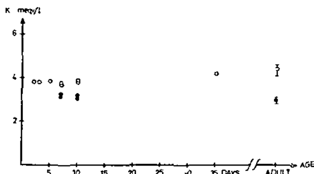


Fig 1 Concentration of potassium in plasma (O) and in CSF (●) plotted against age Anesthetized animals

Thus the present study was undertaken to answer the following questions

1) Is the potassium concentration of the CSF of kittens different from that of adult cats?

2) Is the potency of the homeostatic mechanisms for potassium in CSF at birth comparable to that of the adult animal when elevation of the plasma potassium concentration is induced

Methods

The results were collected from 21 kittens varying in postnatal age between 1 day and 5 weeks and from 6 adult cats

Operative and sampling procedures The animals were anesthetized with 40 mg/kg of Nembutal ip when necessary further anesthesia was added during the experiment The femoral artery and vein were catheterized to obtain arterial blood samples and to allow infusions CSF was obtained from the cisterna magna by using a glass micropipette connected to a thin polyethylene tubing The glass capillary had previously been carefully rinsed with distilled water Use of these micropipettes allowed the taking of samples of CSF large enough to be analysed (more than 20 μl) Arterial blood samples were taken within 2 min after the CSF samples and centrifugated in heparinized capillary tubes Both the CSF and plasma samples were divided into 2 portions and diluted 1 100 with distilled water and the potassium content analyzed with an Eppendorf flame photometer The mean value of the potassium concentration was then calculated for both fluids However if two such portions differed in potassium concentration by more than 0 10 meqv/l the values were discarded

Iv infusions After an initial sampling iv infusion with isotonic (1 14 %) KCl solution was started at a speed of 15 ml/kg b wt and hour in the adults and the older kittens and 10 ml/kg b wt and hour in the younger kittens (see Fig 2) The infusion lasted for 2 h after which new samples of CSF and arterial blood were obtained Attempts to obtain more than 2 liquor samples during 1 expt usually failed with the smallest animal as CSF leakage often occurred through the atlanto-occipital membrane after repeated samplings Experiments showing a final plasma potassium concentration of more than 8 50 meqv/l were discarded since cardiac arrhythmias with circulatory failure often occurred in these cases Three control experiments were performed on small kittens from which CSF samples were taken only after the iv infusion had ended These results did not differ from those presented below In some of the youngest kittens only the initial samples could be obtained thus explaining the small number of "post infusion values" in this age group (Fig 1 and 2)

Per os feeding In 3 kittens 4—5 days old a more prolonged elevation of plasma potassium concentration was obtained by giving isotonic KCl solution through a baby feeding tube In 2 animals the amount of solution given corresponded to 15 of the b wt of the animals while one kitten received only 10 of its b wt. Of these amounts 3/10 were given initially 1/10 every subsequent 2 h After 12 h the animals were anesthetized and samples from and CSF were taken as described previously

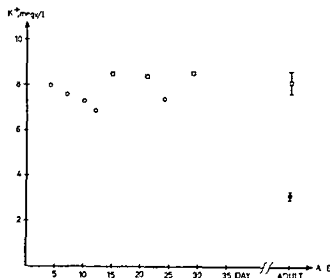


Fig 2 Concentration of potassium in plasma (○ □) and in CSF (● ■) after i v infusion of isotonic KCl at a speed of 10 ml/kg b w /min (round symbols) and 15 ml/kg b w /min (square symbols) plotted against age Anesthetized animals

Results

Fig 1 illustrates the concentrations of potassium in plasma and CSF respectively at various postnatal ages The results show that in anesthetized kittens there are small but significant ($p < 0\,001$) changes in CSF potassium concentration during the postnatal period with a mean value of 3 19 (S E $\pm 0\,07$) meqv/l during the first 2 weeks of postnatal life compared with a mean of 2 95 (S E $\pm 0\,09$) meqv/l the adults The mean value of the plasma potassium concentration was 3 94 S E $\pm 0\,08$) meqv/l in kittens younger than 20 days while the corresponding value for adult cats was 4 40 (S E $\pm 0\,17$) meqv/l *This difference is statistically significant* ($p < 0\,001$)

In order to investigate the possible contribution of anesthetic effects on the values obtained for plasma potassium concentration control experiments were performed on 5 kittens younger than 7 days and on 4 adult cats in which indwelling arterial catheters were inserted during ether narcosis After the anesthesia had been discontinued arterial blood samples were taken when the animals started to move spontaneously The results obtained under these conditions showed a mean plasma potassium concentration of 4 36 (S E $\pm 0\,09$) meqv/l in the kittens compared with a mean value of 4 45 (S E $\pm 0\,08$) in the adults The difference between these values is not statistically significant ($> 0\,1$)

The animals in this control group were then given a large dose of Nembutal (50 mg/kg b wt) i p and in the adult cats a mean plasma potassium concentration of 3 90 (S E $\pm 0\,06$) meqv/l was now obtained The corresponding value in the kittens was 3 78 (S E $\pm 0\,08$) meqv/l This age difference is not significant ($p > 0\,1$)

Fig 2 illustrates the concentrations of potassium in plasma and CSF respectively after intravenous infusion with isotonic KCl for 2 h The results show that in spite of elevations in plasma potassium concentration to values around 8 0 meqv/l the CSF

TABLE I Effect of prolonged elevation of plasma potassium concentration on the CSF potassium concentration in 3 kittens

Postnatal age in days	Amount of isotonic KCl given (% of b wt)	Plasma K^+ conc after 12 h (meqv/l)	CSF K^+ conc after 12 h (meqv/l)
4	10	5 20	3 30
5	15	6 80	3 60
5	15	7 60	3 70

potassium concentration increased only slightly in all ages The largest CSF post infusion value obtained was 3 80 meqv/l in 2 kittens 1 day and 15 days old respectively Also the difference between pre and postinfusion CSF potassium values was found to be slight, yet significantly ($p < 0\ 001$) larger in kittens younger than 14 days of age (mean value 0 38 (S E $\pm$0 07) meqv/l) compared with the adults (mean value 0 18 (S E $\pm$0 04) meqv/l) in spite of higher final plasma potassium values in the latter

Table I presents the final potassium concentrations in plasma and CSF of the three kittens that were given isotonic KCl per os for 12 h It is seen that even after prolonged plasma potassium elevation with a final concentration of 7 60 meqv/l in a 5 day old kitten the CSF potassium value was only 3 70 meqv/l This is comparable to the final CSF potassium values obtained in the acute experiments

Discussion

The number of studies on the development of the homeostatic mechanisms for potassium in the CSF are few Bito and Myers (1970) studied the concentrations of calcium magnesium and potassium ions in plasma and CSF on Rhesus monkeys both during the fetal period and postnatally They found that the distribution ratio CSF/plasma for potassium declined throughout the second half of the gestation period and even during the first week of postnatal life when the adult ratio was obtained In a recent study on sheep (Bradbury *et al* 1972) the CSF potassium concentration was found to decrease during the fetal period with values at term that were close to yet higher than those obtained from the adults Neither of these investigators studied the effects on the CSF produced by changes in plasma potassium concentration

The results of the present study seem to suggest that in anesthetized animals the plasma potassium concentration is lower in young kittens than in adult cats Control experiments investigating the effects of anesthesia do not however confirm this difference

In unanesthetized animals no difference in plasma potassium concentration due to age was found This agrees with earlier studies on e g unanesthetized newborn babies in whom no significant changes seem to occur during postnatal development (Gyllensward *et al* 1957 Izquierdo *et al* 1970) When adult cats were

thetized the plasma potassium concentration decreased to values comparable to those of anesthetized newborn kittens

The difference in plasma potassium concentration obtained between anesthetized and unanesthetized animals agrees with earlier findings indicating that general anesthesia lowers the potassium concentration in plasma in adult animals and humans (Bradbury and Davson 1965 List 1967)

Thus anesthetic effects seem to explain the differences obtained postnatally in plasma potassium concentration in the anesthetized cat This age difference might depend on a changing sensitivity to anesthesia during postnatal development but might also depend on the operative procedures which allow arterial blood sampling almost immediately after the induction of anesthesia in the adults in the youngest kittens more time was needed to perform the operation proceeding the sampling occasion

Fluctuations in plasma potassium concentrations induced by any difference in the effects of anesthesia may render it difficult to determine properties of homeostatic mechanisms for potassium by means of concentration ratios between CSF and plasma as in the study of Bito and Myers (1970) For this reason, the plasma and CSF potassium values have been presented separately in the present study

With regard to the CSF potassium concentration the present results suggest a mean value of 2 95 meqv/l for adult cats which confirms earlier findings (Bito and Davson 1966) The results also suggest a small but significant ($p < 0.001$) decrease in CSF potassium concentration during the postnatal development in the cat This agrees with the results of an ontogenetic study on sheep by Bradbury *et al* (1972)

The CSF potassium values obtained after acute and prolonged elevations of the plasma potassium concentration indicate efficient homeostatic mechanisms for potassium in the CSF not only in adult cats which is in agreement with earlier findings on *e g* dogs (Cooper *et al* 1955) but also in the youngest kittens Still this homeostatic function seems to be slightly less potent in the young animals than in the adults Earlier investigations have shown regional differences in CSF potassium concentration in adult mammals (Ames *et al* 1965 Bito and Davson 1966) CSF collected from exposed choroid plexuses in the adult cat seems to contain a higher concentration of potassium than samples from the cisterna magna (Ames *et al* 1965) In the present study only CSF from the cisterna magna has been considered Thus it cannot be excluded that CSF from the cisterna magna may represent a more freshly secreted CSF pool in the kitten than in the adult cat and that CSF secreted from the choroid plexuses may therefore not show any age differences Nevertheless the nervous tissue lining the cisterna magna will be influenced by CSF presenting small yet significant changes in potassium concentration during the postnatal development in the cat

The results presented here can thus be summarized

1) It is proposed that in anesthetized cats there is a slight but significant decrease in the potassium concentration of CSF obtained from the cisterna magna during postnatal development

9) The results obtained after acute and prolonged elevation of the plasma potassium concentration suggest an efficient potassium barrier function even in the youngest kittens although this function might be slightly less potent than in the adults

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References

AMES A K HIGASHI and F B NESBETT Relation of potassium concentration in choroid plexus fluid to that in plasma *J Physiol* (Lond) 1965 *181* 506—515
BEKAERT J and G DEMEESTER The influence of the infusion of potassium chloride on the cerebrospinal fluid concentration of potassium. *Arch int Physiol* 1951 59 393—394
BITO L Z and H DAVSON Local variations in cerebrospinal fluid composition and its relationship to the composition of the extracellular fluid of the cortex *Exp Neurol* 1966 *14* 266—280
BITO L Z and R E MYERS The ontogenesis of haematoencephalic cation transport processes in the Rhesus monkey *J Physiol* (Lond) 1970 *208* 153—170
BRADBURY M W B and H DAVSON The transport of potassium between blood cerebrospinal fluid and brain *J Physiol* (Lond) 1965 *181* 151—174
BRADBURY M W B J CROWDER S DESAI J M REYNOLDS M REYNOLDS and N R SAUNDERS Electrolytes and water in the brain and cerebrospinal fluid of the foetal sheep and guinea pig *J Physiol* (Lond) 1972 *227* 591—610
BRIGHTMAN M W The distribution within the brain of ferritin injected into cerebrospinal fluid compartments II Parenchymal distribution *Amer J Anat* 1965 *117* 193—220
BRIGHTMAN M W The intracerebral movement of proteins injected into blood and cerebrospinal fluid of mice *Progr Brain Res* 1968 *29* 19—40
COOPER, E S E LECHNER and S BELLET Relations between serum and cerebrospinal fluid electrolytes under normal and abnormal conditions *Am r J Med* 1955 *18* 613—621
GYLLENSWARD C and B JOSEPHSON The development of the serum electrolyte concentration in normal infants and children *Scand J clin Lab Invest* 1957 *9* 21—28
IZQUIERDO J M J FERRERO D ROMEO J L S BADIA and E R WIGIL, Capillary blood potassium chloride sodium calcium phosphorus and alkaline phosphatase in the newborn *Clin chim Acta* 1970 *30* 343—346
LIST W F Serum potassium changes during induction of anaesthesia. *Brit J Anaesth* 1967 *39* 480—484
NICHOLLS J G and S W KUFFLER Extracellular space as a pathway for exchange between blood and neurons in the central nervous system of the leech ionic composition of glial cells and neurons *J Neurophysiol* 1964 *27* 645—671
PAPPAS G T and D P PURPURA Distribution of colloidal particles in extracellular space and synaptic cleft substance of mammalian cerebral cortex *Nature* (Lond) 1966 *210* 1391—1392

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Reflex Vagal Inhibition of Esophageal Motility

By

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The lower smooth muscle part of the esophagus is innervated by inhibitory vagal nerve fibres (Openchowski 1889 Langley 1898) which may produce relaxation of the lower esophageal sphincter during swallowing During this act the vagal non adrenergic fibres described by Martinson (1965) have been shown to mediate a profound relaxation of the stomach as a reflex response to mechanical stimulation of pharyngo-esophageal structures (Abrahamsson and Jansson 1969) The present experiments were aimed to explore whether a similar non adrenergic vagal reflex mechanism can suppress motility also in lower parts of the esophagus

In 7 cats anesthetized with chloralose (50—60 mg/kg) motility was recorded in the lower part of the esophagus In 4 expts pressure recordings were performed utilizing a small balloon filled with 0 2 ml H_2O and placed 1—2 cm proximally to the gastroesophageal sphincter In 3 expts a volume recording technique at constant pressure (Abrahamsson and Jansson 1969) was used instead by means of a 5 cm long balloon that was placed just proximal to the sphincter The pressure recordings were made by a pressure transducer connected to a Rikadenki writer and the volume recordings by a float recorder writing on a kymograph Distensions of proximal parts of the esophagus were made by transient inflation of a 5 cm long latex balloon Mechanical stimulation of the pharynx to induce swallowing was performed with a forceps The right vagus nerve was cut at the cervical level and its distal end put on electrodes for electric stimulation The following drugs were given atropine 0 5 mg/kg i v guanethidine (Ismelin® CIBA) 3—5 mg/kg i v and gallamine thriethiodide (Flaxedil® May & Baker) in repeated doses of 5 mg/kg

Distension of cervical or upper thoracic parts of the esophagus produced a prompt reflex inhibition of lower esophageal (LE) motility whenever rhythmic LE contractions were present while it had no clearcut effect on resting LE pressure in the absence of rhythmic LE contractions (Fig 1) This figure illustrates an analysis of this inhibitory esophageal reflex response in a cat where LE contractions were induced by efferent electric stimulations of the cut right vagus nerve the left vagus being intact The inhibition of the LE pressure waves induced by upper esophageal distension was unaffected by Flaxedil in doses sufficient to eliminate respiratory and other somatomotor movements The inhibitory LE response also persists after administration of guanethidine 5 mg/kg a dose well capable to eliminate reflex

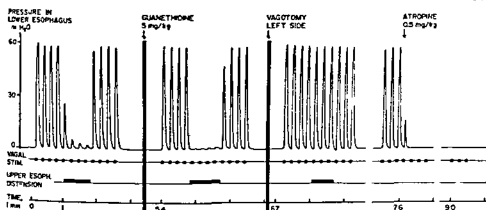


Fig 1 Cat 3 7 kg chloralose anesthesia, immobilization by Flaxedil® right vagus nerve cut Pressure waves in distal esophagus induced by electric stimulation (dots) of the distal end of the cut right vagus (4 Hz 2 ms 6 V during 3 s) 5 times/min Balloon distension (5 ml injection) of the upper part of esophagus reflexly inhibits the contractions in lower esophagus (left panel) This inhibitory response remains after administration of guanethidine 5 mg/kg i v (middle panel) but is abolished by section of the left vagus nerve (right panel) Atropine 0 5 mg/kg i v blocks the vagally induced esophageal contractions

adrenergic inhibition of gastric motility (Jansson and Martinson 1966) The right panel of Fig 1 shows that the reflex inhibition of LE motility is completely abolished by cutting the remaining left vagus nerve The figure also shows that the induced LE pressure waves are themselves eliminated by atropine indicating that they are dependent on a cholinergic vagal mechanism The described vagal non adrenergic inhibitory reflex was also fully evident when LE motility was recorded as volume changes at a fixed pressure load

Mechanical stimulation of the pharynx with associated swallowing movements inhibited motility in lower esophagus in the same manner as distension of the proximal esophagus The effects of guanethidine and vagotomy on the reflex response were the same as in Fig 1

The present study indicates like recent findings in the opossum (Lund and Christensen 1969 Christensen 1970 Tuch and Cohen 1973) that esophageal smooth muscles are controlled by a non adrenergic non cholinergic neural mechanism Furthermore the present results show that the lower smooth muscle part of the esophagus can be reflexly inhibited via this non adrenergic vagal mechanism as induced from mechanoreceptors in the pharynx and the upper esophagus This reflex mechanism appears to be responsible for e g the relaxation of the lower esophageal sphincter that occurs during swallowing and it apparently operates in coordination with the related type of reflex producing relaxation of the reservoir part of the stomach (Abrahamsson and Jansson 1969)

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References

} a d G Jansson Elicitation of reflex vagal relaxation of the stomach from
J phagus in the cat *Acta physiol scand* 1969 *77* 172—1/8
r J Pa terns and origin of some esophageal responses to stretch and electric
a 1 (a r e terology 1970 59 909—916
(and J Martinson Studies on the ganglionic site of action of sympathetic outf
tomach *A ta physiol scand* 1966 *68* 184—19?
J N On inhibitory fibres in the vagus to the end of the oesophagus and the stomach
ysiol (Lond 189 *23* 407—414
o C and J Christensen Electrical stimulation of esophageal smooth muscle and effect
antagonists *Amer J Physiol* 1969 *217* 1369—1374
Martinson J Studies on the efferent vagal control of the stomach *Acta physiol scand* 1
Suppl 255
Ach sk I Ueber die nervosen Vorrichtungen des Magens. *Zbl Physiol* 1889 *3* 1—
Ech A and S Cohen Lower esophageal sphincter relaxation Studies on the neurogenic
inhibitory mechanism *J clin Invest* 1973 *52* 14—20

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hanges in the Electrical Resistivity of Lecithin Coacervates as a Model for Olfactory Transduction

By

G SPERBER

ungenberg de Jong and Saubert (1937) during their studies of coacervates (colloid lutions not miscible with an excess of solvent liquid precipitates) observed that e water contents and consequently the volumes of oleate coacervate phases were ite sensitive to foreign substances of various kinds They made an extensive study this phenomenon measuring however no other property than coacervate volume ey uggested further that this phenomenon might be of relevance to the sense of ell

The present work aims to extend this work by studying the electric resistivity of coacervates this being a biologically more relevant variable Lecithin was chosen tead of oleate since it is an important component of biological membranes

Bungenberg de Jong *et al* (1955) showed that a suspension of egg lecithin forms coacervate in the presence of a suitable amount of butanol The present method s been developed from this

egg lecithin and usually an equimolar amount of an anionic surfa tant (palmitate lauryl phate or tetrapropylene benzene sulphonate as sodium salts the mole ule e ght of lecithin ng taken as 785) was suspended in d stilled water by mechan cal agitation The app opriate ount of a sol ent (approx 1 2 ml n butanol or 0 45 ml ethyl propionate or 1 35 ml ether) s mixed with 10 ml of th s suspens on in a test tube The tube as closed ith a Teflon lined ewcap and provided w th a heat isolating jacket The tube was th n transf rred to a rmostate at 22 0 C and kept overnight. Three ml 1 M NaCl solut on were then added contents of the tube were mixed vigorously and the tube was replaced in the thermostat this stage the coacervate formed an emulsion in the water phase and within some hours arated a an apparently clear liquid phase (there was however some Tyndall scatte ing) top of the water phase with a volume of 3—6 ml and a res stivity of about 100 ohmcm Resistivity measu ements were made at 1000 Hz with a Phil ps 9500 impedance m asurement dge and a micro measuring cell from which the outer bulky jacket had been removed When a stimulus e a mall quantity of an odo ant (20 μl dibutyl ether 20 μl toluene μl n butyl chloride 80 l ethe 50 l methyl isobutyl ketone or half of these doses) was d d to the coacervate (in practice it was added together with the solvent) as a rule the lume d creased and the resi tivity increased (in some cases up to 1000 ohmcm)

The e ponse of a coacervate to a "stimulus was d fined as the difference in resistivity tween the coacervate th and without the "stimulus The "relati e response was def ned the esponse d ded by the re ponse of the same coacervate to a ref rence " timulus l butyl ether as chosen as reference) The 95 % confidence l mits for the relative responses e calc lated according to Goldstein on the basis of quadruplicate sample

The h ghest grad commercial chemical were used without further purif cation except t apropylene benzene sulphonate which was extracted from its contaminat ng m neral salts th a tone water (20 7)

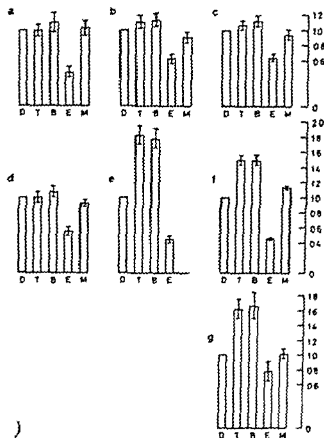


Fig 1 Patterns of response relative to the response to dibutyl ether Columns indicate response to (in a—e) from left to right 20 μl dibutyl ether (D) 20 μl toluene (T) 20 μl n butyl chloride (B) 80 μl ether (E) 50 μl methyl isobutyl ketone (M) or (in f—g) half of these doses The surfactant is none (a) palmitate (b) lauryl sulphate (c) tetra propylene benzene sulphonate (d—g) Solvent is n butanol (a—d) ethyl propionate (e—f) ether (g) Brackets indicate 95 % confidence limit

Representative relative response patterns of some coacervates are shown in Fig 1 In 1a—1d the solvent is n butanol while the surfactant is varied The response patterns are largely similar

In 1d—1g the surfactant is tetrapropylene benzene sulphonate and the solvent is varied These coacervates are somewhat less stable and were therefore also tried with half stimulus doses (1f—1g) It can be seen that the coacervate prepared with ethyl propionate shows a comparatively high sensitivity to toluene and butyl chloride when compared with the butanol coacervates (1e and 1a 1d) and a comparatively low sensitivity to ether when compared with the ether coacervate (1f and 1g half stimulus doses)

Electrophysiological data on the sense of smell permit some inferences about the receptive process Firstly there is evidence (summarised by Ottoson 1970) that the odorant molecules cause a potential change across the plasma membrane of the cilia of the receptor cell presumably by altering membrane permeability

Secondly Gesteland *et al* (1963) studying the action potentials of single receptor cell axons found that each cell has its own individual pattern of sensitivity to odorants No cell was highly specific towards one particular odorant but no two

cells had the same sensitivity pattern This may quite possibly reflect different sen itivity patterns of the receptive membranes of different cells Some authors (Rosen berg *et al* 1968 Davies 1965) have recognised that a model for the receptive process should be able to account for this

On the grounds of the results presented here it is now suggested that if the recep tive membrane contains regions where its lipids have a structure reminiscent of coacervates this would to some extent account for the properties mentioned If an odorant comes into contact with these coacervate regions their resistivity and thus the permeability of the membrane is changed Further if different cells have dif ferent compositions in these regions in analogy with the coacervates whose response patterns are shown in Fig 1d—1g they would respond differentially to different odors in accordance with the properties discussed above

The mechanisms involved and the exact structure of the coacervates are at present rather obscure though there is evidence (Bungenberg de Jong *et al* 1955) that lipid coacervates contain flat bilayer micelles

The proposed model is being further investigated

References

BUNGENBERG DE JONG H G A DE BAKKER and D ANDRIESSE Contributions to the colloid chemistry of phosphatides I *Proc kon n d Akad Wet* Ser B 1955 *58* 238—250
BUNGENBERG DE JONG H G and G G P SAUBERT Kolloidmodelle biologisch r Vorgänge *Protoplasma* (Wien) 1937 *28* 3 9—344
DAVIES J T A theory of the qual ty of odou *J theo Biol* 1965 *8* 1—7
GESTELAND R C J Y LETTVIN W H PITTS and A ROJAS Odor specificities of the fr g olfactory receptors In *Olfa tion and Ta t* Ed Y Zotterman Pergamon Pres 1963 19 34
GOLDSTEIN A *Bio tatist cs* Mac n ll n 1964 a 187
OTTOSON D Electrical s gns of lfactory transducer action In *Taste and sm ll in ...* Ed E W Wolstenholme and J Kn ght Churchill 19 0 343—356
ROSENBERG B T N MISR nd R SWITZER Mechan m of olfactory transduction *Nature* (Lond) 1968 *217* 423—427

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Possible Site of Action of Dopamine in the Hypothalamic Pituitary Control

By

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A dense network of mainly dopamine (DA) nerve terminals has been found in the lateral part of the external layer of the rat median eminence (Fuxe 1964) where the hypothalamic releasing factors (neurosecretory substances) are supposed to enter the hypophyseal portal vessels (Harris 1955) Several studies indicate a possible role of this tuberoinfundibular DA system in the control of the release of pituitary gonadotrophic hormones (Hökfelt and Fuxe 1972 McCann *et al* 1972 Porter *et al* 1972) In the present preliminary study some results are presented which may be of importance for the understanding of the site and mechanism of action of DA in the median eminence

Seven castrated female albino rats (Sprague Dawley b wt 200 g) were used 3 rats received an injection of DA dissolved in Krebs Ringer bicarbonate buffer (10 μg/10 μl during 10 min) into the lateral cerebral ventricle 2 rats were injected intraventricularly with the buffer solution (10 μl) and two rats served as controls 10—15 min after the injection the rats were anesthetized perfused with formalin and glutaraldehyd and processed for electron microscopic analysis At least 15 electron micrographs were taken from the neurohemal contact zone of each rat i e the most superficial zone of the lateral part of the median eminence The surface area of neuronal (nerve endings) and glial (endfeet) elements contacting the basement membrane towards the pericapillary space was measured on all micrographs In this way the percentage of surface area covered by nerve endings in secretion position could be calculated

Nerve endings occupied about 40 % (42.2 ± 6.5) of the surface area in the lateral median eminence (Fig 1) and a similar figure (33.3 % ± 2.2 %) was observed after an intraventricular injection of the buffer solution After injection of DA however almost the entire neurohemal contact zone was covered by glial endfeet and only a few nerve endings (covering 10.5 % ± 4.4 %) reached the basement membrane and thus a secretion position (Fig 2)

The present results demonstrate a potent action of exogenus intraventricularly administered DA on the distribution of glial and neuronal elements in the median eminence It may be speculated that these effects of DA a putative central neurotransmitter are exerted on the glial elements in the median eminence and indirectly

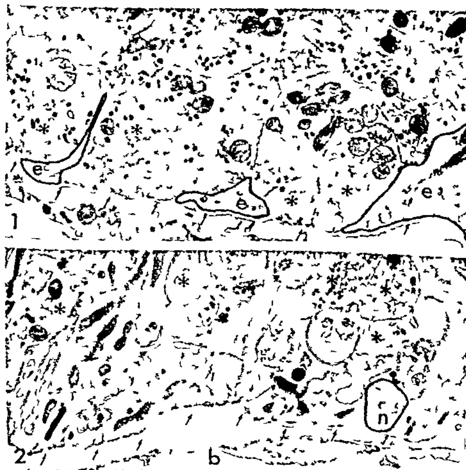


Fig 1 Electron micrograph of the superficial lateral part of the median eminence of a castrated female rat Many nerve endings are seen several of which (asterisks) reach the basement membrane of the surface zone (arrows) These boutons are in a "secretion position" Structures marked by a thick contour line represent glial endfeet (e) Magnification 12 000×

Fig 2 Electron micrograph of the same area of a castrated female rat after injection of 10 μl of DA into the lateral ventricle Only one single nerve ending (n) reaches the basement membrane (arrows) whereas the remaining surface is covered by glial endfeet Most of the nerve endings (asterisks) are localized in a deeper zone Magnification 12 000×

may change the secretion patterns of releasing and/or inhibitory factors from the neurosecretory endings into the portal vessels We are at present investigating the possibility that DA may have similar effects also in other brain areas like the neostriatum since earlier electron microscopic studies have revealed a peculiar lack of typical synaptic contacts in relation to DA boutons in this area (Hökfelt 1968)

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References

FUXE K Cellular localization of monoamines in the median eminence and infundibular stem of some mammals *Z Zellforsch* *1964* *61* 710—724

HARRIS G W *Neuronal control of the pituitary gland* Edward Arnolds (Publishers) Ltd London 1955

HÖKFELT T *In vitro* studies on central and peripheral monoamine neurons at the ultrastructural level *Z Zellforsch* *1968* *91* 1—74

HÖKFELT T and K FUXE On the morphology and the neuroendocrine role of the hypothalamic catecholamine neurons In *Brain Endocrine Interaction Median Eminence Structure and Function* Ed K M KNIGGE D E SCOTT and A WEINDL S Karger Basel 1972 181—223

MCCANN S M P S KALRA A O DONOSO W BISHOP H P G SCHNEIDER C P FAWCETT and L KRULICH The role of monoamines in the control of gonado-tropin and prolactin secretion In *Brain Endocrin Interaction Median Eminence Structure and Function* Ed K M KNIGGE D E SCOTT and A WEINDL S Karger Basel 1972 224—235

PORTER J C I A KAMBERI and J G ONDO *Role* of biogenic amines and cerebrospinal fluid in the neurovascular transmittal of hypophysiotrophic substances In *Brain Endocrine Interaction Median Eminence Structure and Function* Ed K M KNIGGE D E SCOTT and A WEINDL S Karger Basel 1972 245—253